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Meiosis

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Meiosis

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Contents

VII **Preface**
Benavente, R. (Würzburg); Volff, J.-N. (Lyon)

1 **The Meiotic Recombination Hotspots of *Schizosaccharomyces pombe***
Pryce, D.W.; McFarlane, R.J. (Gwynedd)

14 **Meiotic Recombination and Crossovers in Plants**
De Muyt, A.; Mercier, R.; Mézard, C.; Grelon, M. (Versailles)

26 **Meiosis in Cereal Crops: the Grasses are Back**
Martinez-Perez, E. (Sheffield)

43 **Homologue Pairing, Recombination and Segregation in *Caenorhabditis elegans***
Zetka, M. (Montreal)

56 **Homolog Pairing and Segregation in *Drosophila* Meiosis**
McKee, B.D. (Knoxville, Tenn.)

69 **The Mammalian Synaptonemal Complex: A Scaffold and Beyond**
Yang, F.; Wang, P.J. (Philadelphia, Pa.)

81 **The Dance Floor of Meiosis: Evolutionary Conservation of Nuclear Envelope Attachment and Dynamics of Meiotic Telomeres**
Alsheimer, M. (Würzburg)

94 **Cohesin Complexes and Sister Chromatid Cohesion in Mammalian Meiosis**
Suja, J.A.; Barbero, J.L. (Madrid)

117 **Variation in Patterns of Human Meiotic Recombination**
Khil, P.P.; Camerini-Otero, R.D. (Bethesda, Md.)

128 **Maternal Origin of the Human Aneuploidies. Are Homolog Synapsis and Recombination to Blame? Notes (Learned) from the Underbelly**
Garcia-Cruz, R. (Barcelona); Roig, I. (New York, N.Y.); Garcia Caldés, M. (Barcelona)

137 **Inverted Meiosis: The True Bugs as a Model to Study**
Viera, A.; Page, J.; Rufas, J.S. (Madrid)

157 **Author Index**

158 **Subject Index**

Preface

The fifth volume of the book series *Genome Dynamics* is dedicated to 'Meiosis'. Meiosis is a special type of cell division through which haploid cells are generated from a diploid cell and therefore, a key event in the life of sexually reproducing organisms. Meiosis also represents the largest natural source of genetic variability that is a consequence of the recombination and segregation of the maternal and paternal sets of chromosomes.

The field of meiosis research is a rapidly expanding one. Significant progress achieved in recent years has resulted from the use of suitable model systems as well as from the identification and characterization of proteins, many of them meiosis-specific, which are critically involved in key meiotic events. The present volume provides the reader with a series of authoritative review articles summarizing some of the most recent advances in the field of meiosis research. To this end most of the more commonly used model systems have been taken into account and compared.

We wish to express our special thank you to all authors who have contributed to this volume with their excellent review articles and the referees for their expert assistance. Last, but not least, we wish to express our gratitude to Michael Schmid and his team for their invaluable editorial support.

Ricardo Benavente, Würzburg
Jean-Nicolas Volff, Lyon
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The Meiotic Recombination Hotspots of *Schizosaccharomyces pombe*

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Abstract

Meiotic recombination predominantly occurs at genomic loci referred to as recombination hotspots. The fission yeast, *Schizosaccharomyces pombe,* has proved to be an excellent model organism in which to study details of the molecular basis of meiotic recombination hotspot activation. *S. pombe* has a number of different classes of meiotic hotspots, indicating that a single pathway does not confer hotspot activity throughout the genome. The *M26*-related hotspots are a particularly well characterised group of hotspots and details of the molecular activation of *M26*-related hotspots are now coming to light. Moreover, genome-wide DNA array analysis has been applied to the question of meiotic recombination in this organism and we are now starting to get a picture of recombination hotspot distribution on a genome-wide scale.

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Genetic recombination is required for a number of biological processes, including DNA repair, genetic switching and, in meiosis, the correct segregation of homologous chromosomes. The initiation events which result in meiotic recombination products preferentially occur at sites known as recombination hotspots. The features which confer hotspot activity to a site are only partially understood in a limited number of organisms. Recent comparisons between the human and chimpanzee genomic sequences has revealed that meiotic recombination hotspot positions in these two closely related primates are not highly conserved, indicating that meiotic hotspots are evolutionarily unstable [1, 2]. This instability is partly explained by the meiotic recombination hotspot paradox [3] which states that the initiating site (the hotspot) is more frequently converted, and thus, should be transient in evolutionary terms. However, meiotic hotspots are maintained within genomes and must be generated *de novo* more frequently than changes to the genome nucleotide sequence can account for; this indicates that hotspot genesis must be influenced by factors other than DNA sequence alone and it has been postulated that their location is heavily influenced by epigenetic chromosomal features [see 4–6 for recent hotspot reviews].

This review will focus on initiation of meiotic recombination at hotspots of the fission yeast *Schizosaccharomyces pombe*. This model has been widely used to study meiotic processes and has been of particular value for the analysis of meiotic recombination hotspots. Post-initiation events will not be covered, as these have been covered in detail elsewhere [for example, see 7–10].

The *S. pombe M26, CRE* and $M26_{cs}$ Hotspots

Sequence Specificity and DNA Binding Proteins

The *ade6* gene of *S. pombe* is widely used as a genetic marker. Original studies of *ade6* isolated 394 mutant alleles, one of which, *ade6-M26*, was unique in that it generated a 13-fold higher incidence of prototrophic recombinants during intragenic crosses relative to other *ade6* mutant alleles [11]. Gutz extended this analysis to demonstrate that *ade6-M26* was predominantly converted to wild-type in crosses between strains carrying the *M26* and wild-type alleles of *ade6* [11]. From these observations it was concluded that *ade6-M26* was a hotspot for meiotic gene conversion. Later studies established that *ade6-M26* was also a hotspot for meiotic crossing over and displayed no statistically discernable mitotic recombination hotspot activity [12–14].

Sequencing of the *ade6-M26* allele revealed a G to T transversion, within the *ade6* open reading frame (at nucleotide 136 taking the A of the ATG start codon as nucleotide 1) [14, 15]. It was later determined that the *ade6-M26* hotspot activity was dependent upon a heptanucleotide sequence (5′-ATGACGT-3′) generated by this transversion [16]. The *M26* heptamer is bound by the Atf1·Pcr1 stress response heterodimeric transcription factor (Atf1 is also known as Gad7 and Mts1; Pcr1 is also known as Mts2) [17]. Atf1·Pcr1, and the kinase pathway required for its activation, are absolutely required for *ade6-M26* hotspot activation [18–21].

Early work indicated that *M26* was active within the *ade6* open reading frame in a position- and orientation-independent fashion [22]; later studies challenged this and found that at some locations within *ade6* the *M26* heptamer was not an active hotspot [23]. This suggested that broader features of sequence and/or chromatin context influence whether or not the *M26* heptamer can confer hotspot activity to a site. Atf1·Pcr1 binds to a group of *M26*-like elements, known as cAMP response elements *(CREs)* and these were shown to also serve as meiotic recombination hotspots [21]. Recent studies have revealed that a broader sequence motif can influence whether or not a site can confer hotspot activity. An optimal consensus sequence has now been generated indicating that an 18-bp sequence motif is important, 5′-GNVTATGACGTCATNBNC-3′ (V is A, C or G; B is C, G or T; N is any nucleotide), which contains the *M26* heptamer at its core (underlined) [23]; this sequence is termed the $M26_{cs}$ (*M26* consensus sequence; this term shall be used generically for any *M26*-related sequence). Regions containing the optimal sequence can increase the hotspot activity of the *M26* heptamer by up to 15-fold relative to *ade6-M26* [22, 24].

The study of *M26* and $M26_{cs}$ hotspot activation has largely been carried out using artificially introduced sequences. The question of whether *M26*/$M26_{cs}$ sequences could act as natural recombination hotspots was recently answered when 15 naturally occurring *M26*-related sequences were analysed for meiotic double-stranded DNA break (DSB) activity, a physical indicator of meiotic recombination hotspot activity (see below). Ten of these proved to have associated measurable DSBs, and one, within the *cds1* gene, was used to demonstrate that a natural *M26*-related sequence does function as a meiotic recombination hotspot [24]. Furthermore, naturally occurring *M26* sites at the *ctt1* locus exhibit a change in meiotic chromatin structure, indicative of meiotic recombination activation (see below) [19]. These data provide the first sequence-specific predictor of naturally occurring eukaryotic meiosis-specific recombination hotspots. It remains unclear whether the sites with no measurable DSB activity serve as hotspots.

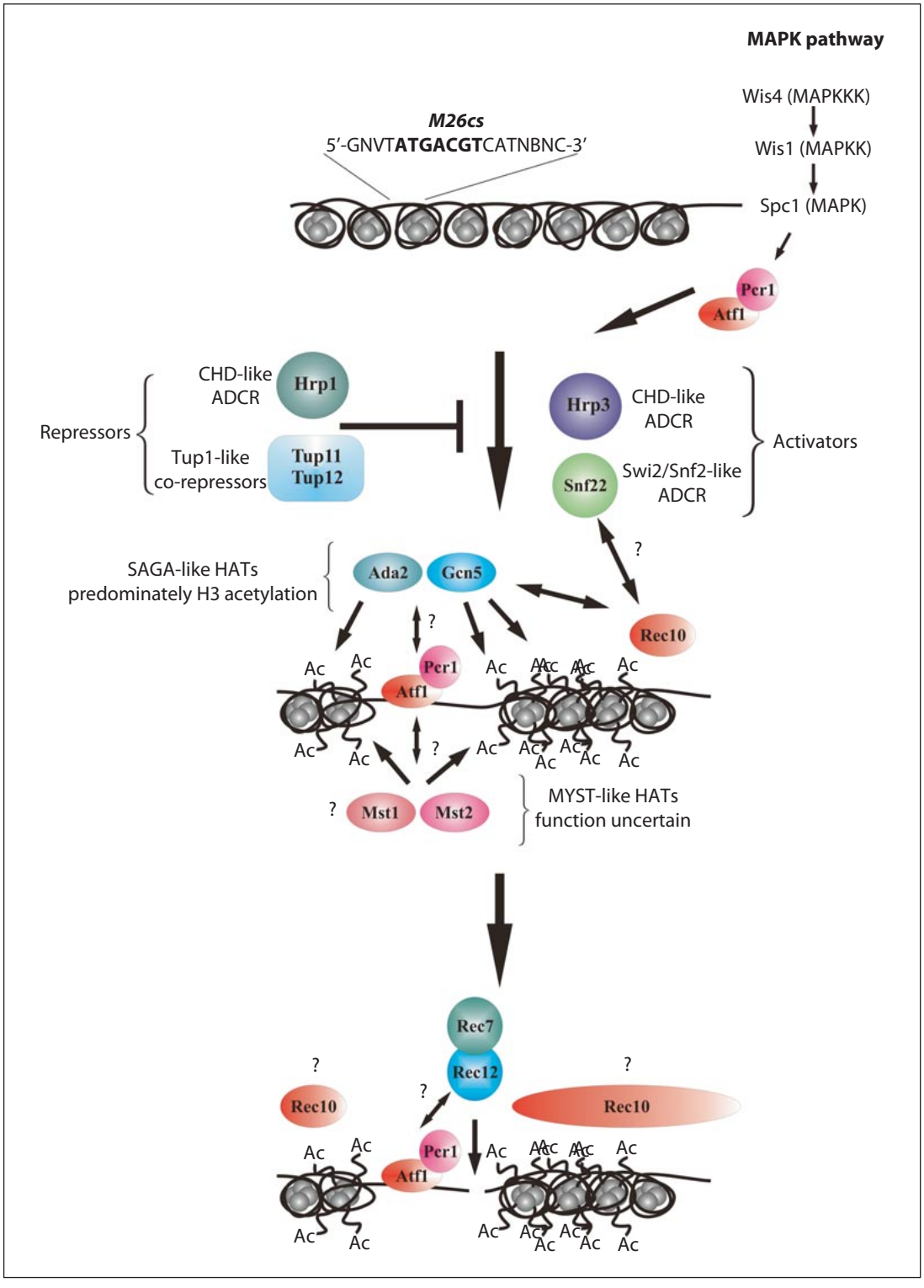
Interestingly, artificial tethering of Atf1 to the *ade6* gene confers hotspot activity [Wahls and co-workers, unpublished], and some $M26_{cs}$ hotspots are Atf1-dependent, Pcr1-independent [24]. This leads to the possibility that Pcr1 serves only to provide DNA binding capability to Atf1 for some $M26_{cs}$ sites and for others it is dispensable. It is possible that Atf1·Atf1 homodimers are capable of binding to $M26_{cs}$ sites containing internal palindromes and that Pcr1 is only required when sites are non-palindromic [Wahls and co-workers, unpublished].

The majority of meiotic recombination in *S. pombe* is dependent upon the topoisomerase II-like protein Rec12 [25]. This highly conserved protein (known as Spo11 in other organisms) generates DSBs, which are proposed to initiate recombination at the DNA level [for review, see 9]. Rec12-dependent DSBs have been detected associated with *M26* [26]. The intensity of the DSBs and their exact position, relative to the *M26* heptamer, differ dependent upon the context of the heptamer [26]. However, at given *M26* locations DSB intensity appears to correlate well with the level of gene conversion [26]. This has resulted in a model in which *M26* serves as a site for Rec12 recruitment (fig. 1). However, no direct associations between Atf1·Pcr1 and Rec12, or other proteins known to be required for the initiation of recombination, have yet been found.

M26: *Chromosomal Architecture*

It has been demonstrated that the *M26* heptamer can be active when placed at different locations within the genome [22, 27, 28]. However, some transplacements of segments of DNA containing *ade6-M26* result in no hotspot activity [27, 28] and the *ade6-M26* allele exhibits no hotspot activity when present on an autonomously replicating plasmid molecule [27]. These factors indicate that chromosomal architecture plays a critical role in whether the *M26* heptamer (or related $M26_{cs}$ sequences) confers hotspot specificity.

The positioning of nucleosomes for the *ade6* locus has been determined by micrococcal nuclease digestion [29]. During mitotic growth the nucleosomes form a regular array at *ade6*. On entry into meiosis the positioning of the nucleosomes in the vicinity


MAPK pathway
Wis4 (MAPKKK)
Wis1 (MAPKK)
Spc1 (MAPK)
M26cs
5′-GNVTATGACGTCATNBNC-3′
Per1
Atf1
Repressors
CHD-like ADCR
Hrp1
Tup1-like co-repressors
Tup11 Tup12
Hrp3
CHD-like ADCR
Snf22
Swi2/Snf2-like ADCR
Activators
SAGA-like HATs predominately H3 acetylation
Ada2
Gcn5
Rec10
Ac
MYST-like HATs function uncertain
Mst1
Mst2
Rec7
Rec12
Rec10

of the *M26* heptamer alters (approximately 600 bp flanking the *M26* heptamer). This is consistent with active remodelling of chromatin which presumably makes the DNA at this site more accessible to the proteins which initiate and mediate recombination, such as Rec12 [29]. This phenomenon has been termed the chromatin transition.

Whilst the chromatin transition is very prominent on entry into meiosis it is also apparent when cells are switched to medium depleted of nitrogen and so occurs to a limited degree when cells make the transition from mitotic proliferation into a pre-meiotic state [19]. The transition is also apparent in response to osmotic stress, but not salt or oxidative stress [30]. There is also a requirement for ploidy-independent heterozygosity of the mating type locus for the full chromatin transition to occur [19]. Mating type heterozygosity is required for Mei3 expression which, in combination with Mei2 [31], is required for meiotic entry and pre-meiotic DNA synthesis; both Mei2 and Mei3 are required for a full chromatin transition at *ade6-M26* [19].

Recent work has revealed a complex regulatory network controlling the chromatin transition at *ade6-M26* (table 1; fig. 1) [32, Ohta and co-workers, unpublished]. ATP-dependent chromatin remodelling factors (ADCRs) function in both agonistic and antagonistic fashion. The Swi2/Snf2 family protein Snf22 is absolutely required for the chromatin transition; loss of Snf22 function also results in a loss of almost all *M26* hotspot activity with little or no effect on non-hotspot activity [32]. Two CHD (chromodomain helicase DNA binding)-like ADCRs, Hrp1 and Hrp3, appear to play opposing roles in *M26* hotspot regulation [Ohta and co-workers, unpublished]. Hrp1 acts to repress the chromatin transition and loss of Hrp1 results in a constitutively open chromatin configuration at *M26* and the *hrp1Δ* mutant has hotspot activity similar to the wild-type [Ohta and co-workers, unpublished]. This repression is similar to the activity of the Tup-like transcriptional co-repressors, Tup11 and Tup12, which are also required to repress the chromatin transition at *M26* [33]. In contrast, loss of Hrp3 results in a loss of both a measurable chromatin transition and hotspot-specific recombination, with no loss of non-hotspot recombination, indicating there is a correlation between hotspot activation and the Hrp3-dependent chromatin transition.

Associated with the chromatin transition is a hyper-acetylation of histones H3 and H4 within nucleosomes at *M26* (fig. 1) [32, Ohta and co-workers, unpublished]. Histone acetylation is mediated by histone acetyl transferases (HATs) and two families

Fig. 1. Model for the activation of the *ade6-M26* meiotic recombination hotspot. Nucleosomes of mitotic chromatin (top) are regularly arrayed throughout the *ade6-M26* allele ($M26_{cs}$ sequence is shown; top). On entry to meiosis the chromatin associated with the hotspot becomes hyperacetylated and the nucleosomes are remodeled. The acetylation and remodeling is mediated/suppressed by a number of different *trans* activating proteins (see main text for more detail). Concomitant with this the Atf1·Pcr1 heterodimer is activated by the MAPK pathway (top right) and the Atf1·Pcr1 heterodimer then binds to the *ade6-M26* heptamer (bold nucleotides within the $M26_{cs}$ sequence; top). This process is thought to culminate in a more open chromatin configuration which makes the DNA more directly accessible for recombination initiating proteins, such as Rec12 (bottom). The chromatin transition is also influenced by the axial structure protein, Rec10, in ways that remain unclear. Question marks indicate interactions which are currently hypothetical. Refer to main text for a detailed description.

Table 1. A list of mutants defective in aspects of *ade6-M26* dynamics

Mutant	*ade6-M26* recombination status[a]	*ade6-M375* recombination status[a]	*ade6-M26* hotspot activity[b]	*ade6-M26* meiotic chromatin transition	*ade6-M26* meiotic chromatin acetylation status	*ade6-M26* DSB status	*ade6-M26*-associated meiotic-specific transcript
Wt	Normal	Normal	Normal [11]	Present [30]	H3/H4 hyper Ac [32]	Full [26]	Induced[c]
atf1Δ	Reduced	Normal	Lost [18]	Lost [32]	H3/H4 Ac both down [32]	N.D.	
pcr1Δ	Reduced	Normal	Lost [18]	Lost [32]	N.D.	Lost [26]	N.D.
gcn5Δ	Reduced	Normal	Partial loss [32]	Reduced/ delayed [32]	H3/H4 Ac both down (H3 only partly) [32]	Reduced/ delayed[c]	Not induced[c]
ada2Δ	Reduced	Normal	Partial loss [34]	Reduced[c]	H3/H4 Ac both down (H3 only partly) [32]	Reduced[c]	Reduced[c]
snf22Δ	Reduced	Normal	Lost [33]	Lost [32]	H3/H4 Ac both down [32]	Reduced[c]	Reduced[c]
hrp1Δ	Normal	Normal	Normal [32]	Constitutively open[c]	H3 Ac higher/ H4 normal [32]	N.D.	N.D.
hrp3Δ	Reduced	Normal	Partial loss[c]	Reduced[c]	H3/H4 Ac both down [32]	Reduced[c]	Reduced[c]
mst2Δ	Reduced	Reduced	Normal[c]	Reduced[c]	H3/H4 hyper Ac [32]	N.D.	N.D.
tup11Δ tup12Δ	N.D.	N.D.	N.D.	Constitutively open [33]	N.D.	N.D.	N.D.
spc1Δ	Reduced	Normal	Lost [20, 21]	Lost [25]	N.D.	N.D.	N.D.
wis1Δ	Reduced	Normal	Lost [20, 21]	Lost [25]	N.D.	N.D.	N.D.
rec10-144	Reduced	Reduced	Partial loss [41]	N.D.	N.D.	N.D.	N.D.
mei2Δ	N.D	N.D.	N.D.	Reduced [19]	N.D.	N.D.	N.D.
mei3Δ	N.D.	N.D.	N.D.	Reduced [19]	N.D.	N.D.	N.D.

Table 1. (continued)

Mutant	*ade6-M26* recombination status[a]	*ade6-M375* recombination status[a]	*ade6-M26* hotspot activity[b]	*ade6-M26* meiotic chromatin transition	*ade6-M26* meiotic chromatin acetylation status	*ade6-M26* DSB status	*ade6-M26*-associated meiotic-specific transcript
hsk1-89	Reduced	N.D.	N.D.	Reduced/ delayed [43]	N.D.	N.D.	N.D.
rad2*Δ*	Reduced[d]	Normal[e]	Partial loss [44]	N.D.	N.D.	N.D.	N.D.

[a]Derived from two factor crosses with different marker alleles.
[b]The ratio of the recombination frequency obtained for *ade6-M26* / the recombination frequency obtained for *ade6-M375* (a control allele generated by a G to T mutation in the codon adjacent to the *ade6-M26* mutation).
[c]Hirota K, Mizuno KI, Shibata T, Ohta K: Unpublished data (submitted).
[d]*ade6-3049* (an allele of *ade6* which contains an *M26* heptamer; *ade6-M26* was not tested).
[e]*ade6-3057* (a non-hotspot control allele for *ade6-3049*).

of HAT have been implicated in mediating histone H3 and H4 acetylation at *M26*. These are the SAGA family of HATs, which in *S. pombe* includes Gcn5 and Ada2, and the MYST family, which in *S. pombe* includes Mst2 [34]. Null mutations in both *gcn5* and *ada2* reduce *M26* hotspot activity, with no alteration in basal recombination frequency, indicating hotspot specificity. However, whilst there is a significant reduction of histone H3 acetylation in the *gcn5Δ* and *ada2Δ* mutants, there is only a minor reduction in histone H4 hyperacetylation patterns. This suggests that there are other HAT activities responsible for a significant proportion of histone H4 acetylation in *S. pombe*; loss of Mst2 function alone gives no significant reduction of either H3 or H4 acetylation, suggesting this is not the alternative HAT activity required in the absence of Gcn5 and Ada2. In *S. cerevisiae* the NuA4 HAT exhibits histone H4 specificity [35], yet to date mutants of the *S. pombe* homologue of the Esa1 catalytic subunit, Mst1, have not been tested as this protein is essential and conditional mutants have not been generated. Whilst the *mst2Δ* mutant has no detectable chromatin transition at *M26*, it does exhibit a reduction in both basal and hotspot allele recombination uniformly indicating that the loss of recombination function is not hotspot-specific and a measurable hotspot activity is retained [Ohta and co-workers, unpublished]. *snf22Δ* mutants have reduced histone H3 and H4 acetylation on meiotic entry, suggesting that the chromatin remodelling by Snf22 precedes or is concomitant with *M26* nucleosome hyperacetylation.

The Atf1·Pcr1 activator of *M26* is required for the chromatin transition and the hyperacetylation of histones H3 and H4 [32]. The kinase pathway required for activation of Atf1·Pcr1 is also required for the chromatin transition to occur normally [32].

However, it remains unknown if there are direct interactions between Atf1·Pcr1 and/or the *M26* DNA with the chromatin regulation machinery.

Recent developments have shown that a specific mRNA transcript is initiated 2 bp upstream of the *M26* heptamer sequence within the *ade6-M26* allele [Ohta and co-workers, unpublished]. This transcript is produced pre-meiotically, but is significantly up-regulated on meiotic entry. A similar short transcript, which has a different initiation site, is associated with *M26* in response to osmotic stress [30]. The up-regulation of the meiotically induced transcript is dependent upon the factors required for chromatin remodelling and histone acetylation, but they are not required for the pre-meiotic transcription from this site [Ohta and co-workers, unpublished]. From this it has been postulated that RNA polymerase II is required for *M26* activation. Deletion of the *ade6* promoter region has been shown to result in the loss of *M26* hotspot activity, but interpretation of this observation is complicated by the fact that promoter loss can alter the genetic read out and potentially significantly alter the chromatin context in which *M26* is embedded [36]. Up-regulation of *ade6* under the ADH promoter results in elevated *M26* hotspot activity, indicating that processes associated with RNA polymerase II-mediated transcription influence *M26* hotspot activity; however, the exact role(s), if any, played by RNA polymerase II and/or the nascent RNA remains unclear [37].

During meiosis, unique changes occur to chromatin architecture, many of which are thought to be associated with the pairing of homologous chromosomes [reviewed in 38]. In *S. pombe* this includes the formation of meiosis-specific proteinaceous, linear structures which are associated with the chromosomes, termed linear elements (LinEs) [reviewed in 38, 39]. The exact function of these structures remains unknown, but they are related to the pre-synaptic axial structures found in other organisms. One of the main components of these chromosomal structures is the Rec10 protein, which is related to the *S. cerevisiae* lateral element protein Red1 [40]. Loss of Rec10 function dramatically reduces recombination throughout the genome; however, specific hypermorphic mutants of *rec10* reduce the hotspot activity of some *M26*-containing hotspots, but not all [41]. The molecular basis for why some, but not other, *M26* hotspots require Rec10 function(s) remains unknown. However, there is sufficient evidence to speculate that it is related to the extent of the nucleosome alterations during the chromatin transition [41]. This indicates that higher order chromatin regulators potentially have a significant influence over the activation of hotspots.

Association of M26 *Activity with Pre-meiotic DNA Replication*

In *S. cerevisiae* the chromatin transition at meiotic recombination hotspots is dependent upon pre-meiotic S-phase [42]. To date this has not been explored carefully in *S. pombe*, however, the Hsk1 kinase is required for a measurable chromatin transition to occur at the *ade6-M26* hotspot [43]. Hsk1 is the *S. pombe* homologue of the Cdc7 kinase, which is required for mitotic DNA replication; in *S. pombe* the analysis of *M26* dynamics has been studied in an *hsk1* conditional mutant in which pre-meiotic DNA replication does occur, albeit with altered kinetics [43]. *ade6-M26* meiotic recombi-

nation is reduced in the *hsk1*-defective cells, but it remains unknown whether or not this is hotspot-specific as non-hotspot basal recombination was not measured [43].

A further possible link between $M26_{cs}$ hotspot activity and pre-meiotic DNA replication comes from the fact that the hotspot activity of the *ade6–3049* allele (an *M26* heptamer hotspot within *ade6*) is reduced in a mutant defective in the FEN-1 flap endonuclease homologue, Rad2 (Fen1) [44]. FEN-1 is required for Okazaki fragment processing during DNA replication [45]. This observation indicates that there might be a link between lagging strand processing and hotspot activity; however, *rad2Δ* mutants exhibit no measurable defect in the timing or kinetics of pre-meiotic DNA replication [44] and so other roles for the Rad2 (Fen1) flap endonuclease cannot be ruled out.

Other Recombination Hotspots of *S. pombe*

mbs1 *and* mbs2

Physical analysis of DSB sites in *S. pombe* has revealed a number of prominent break sites [25, 46]. Two of these, meiotic break site 1 (*mbs1*) and meiotic break site 2 (*mbs2*) have been found to be located within large intergenic regions [46]. *mbs1/2* are Rec12-dependent, correlate well with the sites of Rec12 binding (see below) and serve as hotspots for gene conversion which are associated with elevated crossover frequencies [47]. Whilst the sites of *mbs1* have been resolved down to a 2.1 kb region, a detailed characterisation of this site remains to be completed. Interestingly, whilst elevated crossovers are associated with *mbs1*, adjacent regions, which are devoid of measurable prominent DSBs, have almost equal crossover frequencies, indicating a more complex control of crossover regulation.

ura4::aim

The *ura4::aim* (artificially introduced marker; also called *ura4A*) hotspot was generated when the *ura4* gene was inserted in a site adjacent to *ade6* to create a linked marker for genetic analysis [36]. It transpired that *ura4::aim* generated an independent meiotic recombination hotspot [36, 48], with high conversion frequencies of 18% (compared to a maximum of 7.5% for *ade6-M26*) [48]. The *ura4::aim* hotspot activity is dependent on a 15-bp region of DNA of unknown origin located at the *ura4* insertion junction. The molecular nature of the *ura4::aim* hotspot remains unknown. *ura4::aim* exhibits cross talk with the *ade6-M26* hotspot; when both are located within 15 kb of each other they have a reciprocal negative effect on their respective hotspot activities. The molecular basis of this inter-hotspot inhibition remains unknown. Studies on *ura4::aim* have also revealed some interesting features of hotspot biology [48]. Firstly, there is a mating type conversion bias; *S. pombe* has two mating types, h^+ and h^-, when *ura4::aim* enters with the h^+ configuration it is preferentially converted; this might suggest that h^+ cells have a chromatin configuration more amenable to conversion. Secondly, studying the *ura4::aim* hotspot has

confirmed the existence of map expansion, in which recombination frequencies obtained for distally placed markers in two factor crosses are greater than the sum of recombination frequencies obtained using internal marker pairs covering the same region [48, 49]; the existence of map expansion had been previously questioned [50].

M-pal

Whilst palindrome sequences are not commonly found within the *S. pombe* genome, an artificially inserted palindrome, M-pal can serve as a Rec12-independent meiotic recombination hotspot [51]. The initiation of recombination is dependent upon the MRN complex and it is postulated that this complex is capable of converting cruciform structures generated by the M-pal sequence into breaks via a hairpin-specific nuclease activity [51]. Whilst this might serve as an important mechanism for the elimination of palindromes which may result in deleterious chromosomal breakage, the relevance to normal meiotic pathways remains unclear.

Genome Wide Analysis of Meiotic Rec12 Binding

Recently, two groups have applied chromatin immunoprecipitation and microarray technology to the study of DSB sites throughout the 12.6-Mb genome of *S. pombe* by identifying the sites at which a tagged version of the Rec12 protein is covalently linked to DNA [52, Kohli and co-workers, unpublished]. These two studies yielded both common and conflicting findings and it is assumed that the differences are based on the fact that one employed formaldehyde cross linking [Kohli and co-workers, unpublished] and the other did not (and so only identified sites at which Rec12 was covalently linked to the DNA via natural covalent linkage during the generation of the DSB) [52]. As Rec12 is required for DSB formation it is assumed that identifying the sites of meiotic Rec12 binding will serve as a good indicator of meiotic recombination hotspots. Indeed, this idea is supported by the fact that the location of Rec12 association with a 1.8-Mb region of chromosome I correlates well with physical analyses of DSB sites within this same region [52].

One study (no cross linking) identified in the region of 350 prominent Rec12 sites (353 in the haploid and 340 in the diploid), whilst the other study (with cross linking) identified 144 prominent Rec12 binding sites. No centromeric or telomeric Rec12-association was identified when cross linking was not employed, but a strong centromeric association was found with cross linking, which may indicate other meiotic functions for Rec12. One study found a positive correlation with the binding of the Rec8 meiosis-specific cohesin [Kohli and co-workers, unpublished] and the other did not [52]. An inverse correlation between DSB sites and the Rec8 meiotic cohesin is consistent with a model from a study in the budding yeast, which predicts that prominent DSBs form within loops emanating from an axial core which contains cohesin protein [53]. The main correlation to be found in both studies was that prominent

Rec12 loading sites are more frequently located within larger intergenic regions. This suggests that features of these regions, possibly transcription factor binding sites, such as $M26_{cs}$, provide a favourable chromatin environment for Rec12 loading. Consistent with this, the *mbs1* and *mbs2* sites were found in large intergenic regions [46]. Neither study found any correlation between origins of DNA replication, which have recently been defined for meiosis [54]. Interestingly, crossovers appear to be relatively evenly distributed throughout the *S. pombe* genome, despite the fact there is no genetic interference evident is *S. pombe* [reviewed in 55]; however, the study which did not employ cross linking found Rec12 loading sites within the *S. pombe* genome to be frequently spaced approximately 50–100 kb apart, indicating that there are important features of recombination control that we currently have a very poor understanding of.

Closing Remarks

Work in the fission yeast has started to shed light on the complex nature of the biology of meiotic recombination hotspots. It provides us with a system which has features in common with humans; for example, human recombination hotspots, which have been determined by linkage disequilibrium and sperm typing, have interhotspot distances similar to the distances between *S. pombe* Rec12 binding sites. Whilst much progress has been made, many unanswered questions remain. However, we do know that hotspot activity is conferred by a complex series of factors operating at a number of levels within the architecture of a meiotic chromosome. Further studies in this amenable system will enable us to unravel these factors and how they interplay with one another.

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Meiotic Recombination and Crossovers in Plants

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Abstract

Efforts have been made in recent years to clarify molecular meiotic processes in a large variety of higher eukaryotes. In plants, such studies have enjoyed a boom in the last years with the use of *Arabidopsis thaliana* together with maize, rice and tomato as model systems. Owing to direct and reverse genetic screens, an increasing number of genes involved in meiosis have been characterized in plants. In parallel, the improvement of cytological and genetical tools has allowed a precise description of meiotic recombination events. Thus, it appears that meiotic studies in plants are reaching a new stage and can provide new insights into meiotic recombination mechanisms. In this review, we intend to give an overview of these recent advances in the understanding of meiotic recombination in plants. Copyright 2009 © S. Karger AG, Basel

Meiosis is of particular interest in biology because it generates the haploid cells that are required for the sexual reproduction process, and is the physical basis of Mendelian genetic inheritance. Recombination is one of the key events in meiosis. It gives rise to crossovers (reciprocal exchange of DNA fragments between homologous chromosomes), which are essential for the correct segregation of homologous chromosomes during the first meiotic division, ensuring the linking of homologous chromosomes (bivalent formation, [1]). Crossovers are also important because they are used to construct genetic maps.

Model of Meiotic Recombination and Meiotic Recombination Markers

The working model of meiotic recombination is summarized in figure 1. According to this model, meiotic recombination is initiated by the programmed formation of DNA Double-Strand Breaks (DSBs), which are later resected to generate 3′ single stranded DNA ends that drive DNA repair, using the homologous chromosome as a template.

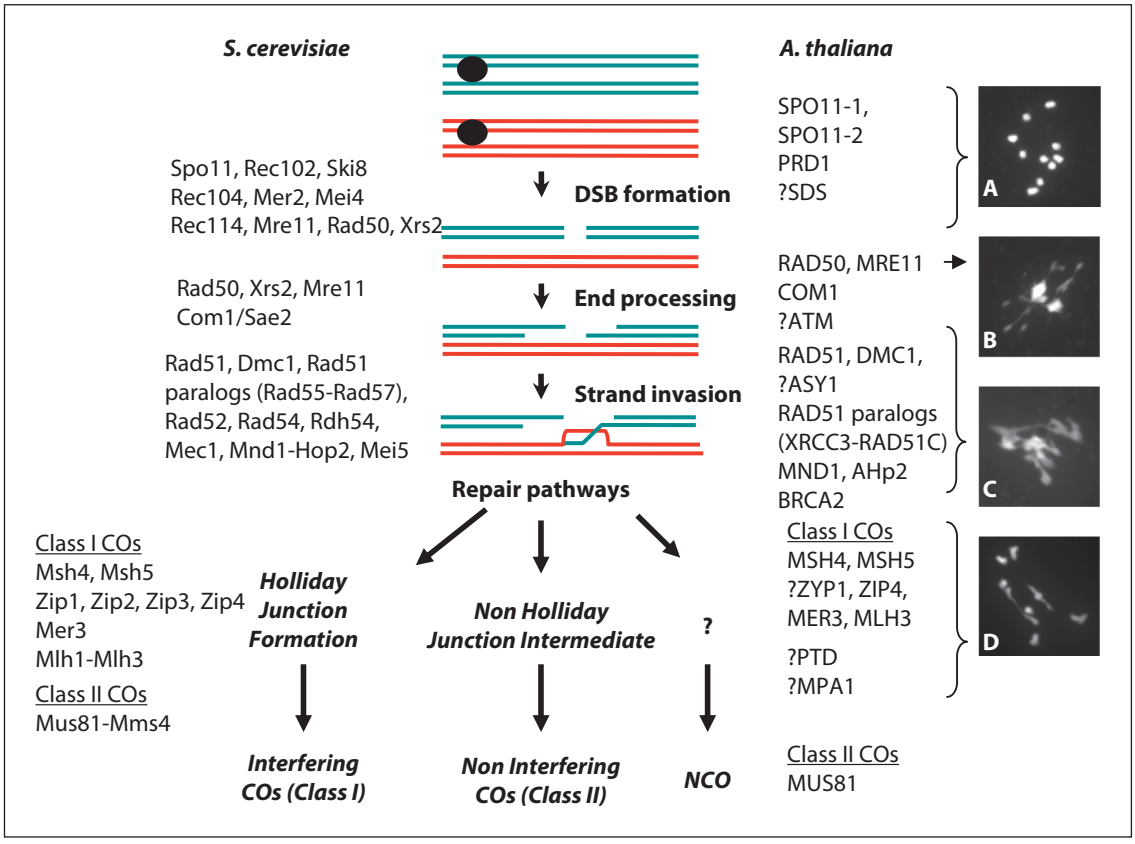

Fig. 1. Schematic representation of the different steps of meiotic recombination. For each step, proteins known to be involved in that step in *S. cerevisiae* or in *A. thaliana* are indicated. When such assignment is only hypothetical, a question mark has been added. The phenotypes at first meiotic metaphase of *A. thaliana* mutants disrupted in any of these steps are also indicated (**A**–**D**) and should be compared to the wild-type situation shown in figure 2A.

Several markers of meiotic progression are now available for plants (fig. 2), but others are needed. For example, it is not yet possible to visualize DSBs directly. The number of meiotic DSBs is therefore estimated indirectly by quantifying DSB repair sites via the immunodetection of RecA-like recombinases (RAD51 and DMC1) or the detection of Early Nodules (ENs) of recombination on 2D chromosome spreads viewed in an electron microscope [2] (fig. 2B, C, table 1).

One of the final products of recombination – crossovers (COs) – can be scored in different ways: (i) classical genetic analysis of segregation of markers in the offspring, (ii) genetic analysis using the very powerful newly developed visual assay on *Arabidopsis* tetrads [3], (iii) chiasma counting [4], (iv) counting of Late Nodules (LNs), which are thought to correspond to CO sites, at pachytene ([2], fig. 2D) or (v) immunostaining of MLH1, which acts as a marker of a subset of COs (class I COs, see below and [5]). A wide range of recombination intermediates and products that

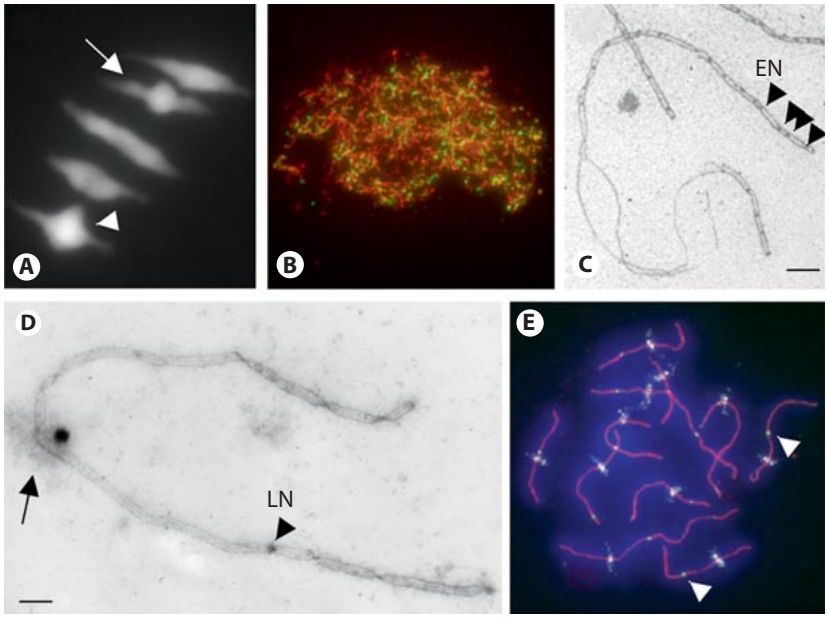

Fig. 2. Meiotic recombination markers in plants. **A** DAPI staining of an *Arabidopsis thaliana* pollen mother cell at metaphase I. A bivalent with a single chiasma is indicated by an arrow and a bivalent with two chiasmata is indicated by an arrowhead. **B** Multiple immunofluorescence of an *Arabidopsis thaliana* pollen mother cell spread, using anti-ASY1 (red) and anti-DMC1 (green) antibodies. **C** A tomato synaptonemal complex (SC) at zygotene. Some early nodules (EN) are indicated by arrowheads. From Lorinda Anderson and Stephen Stack. Bar = 1 μm. **D** A tomato SC at mid-late pachytene. A late nodule (LN) is indicated by an arrowhead. The fuzzy kinetochore is indicated by an arrow. From Lorinda Anderson and Stephen Stack. Bar = 1 μm. **E** Multiple immunofluorescence of a tomato pollen mother cell spread, using anti-MLH1 (green), anti-SMC1 (red), anti-CENPC (grey) antibodies and DAPI (blue). From Franck Lhuissier. Some MLH1 foci are indicated by arrowheads.

cannot yet be cytologically scored are formed between DSB formation/strand invasion and CO formation.

Distribution of Recombination Events

In plants, as in other eukaryotes, the total size of the genetic map varies considerably between species (table 1). However, CO rates, measured in cM/Mb are roughly inversely proportional to the genome size (table 1). Thus, the number of chiasmata does not increase proportionally with the genome size, consistent with the existence of controls ensuring at least one CO per bivalent but limiting the total number of COs.

As in all eukaryotes, the distribution of COs on the chromosomes is not uniform in plants (reviewed in [6]). This heterogeneous distribution results from several layers of control, including interference. This phenomenon was described by Sturtevant in 1915, as follows: 'The occurrence of one crossing-over in a given chromosome pair

Table 1. An overview of plant recombination data

Organisms	Genome size Mb	Haploid chr. number	Genetic size cM	cM/Mb	ENs or RAD51/DMC1 foci	LNs or chiasma number	CO/DSB[f]
A. thaliana	120 [74]	5	470 [75]	3.9	220 [14, 53]	9.2 [76]	24
M. truncatula	475[a]	8	1,125 [77]	2.4			
O. sativa	430 [78]	12	1,530 [79]	3.55			
L. japonicus	475[a]	6	500 [80]	1.05			
P. trichocarpa	485 [81]	19	2,500 [82]	5.2			
L. esculentum	824[a]	12	1,469 [83]	1.8	292[e]	22 [84]	13
E. guineensis	1,750 [85]	16	1,743 [86]	1			
Z. mais	2,365[b]	10	1,729[b]	0.73	500 [87]	21.9 [88]	23
S. cereale	8,300[a]	7	921[c]	0.11			
A. fistulosum	9,900[a]	8			669 [89]	15 [90]	44
A. sativa	11,400[a]	21	2,932 [91]	0.25			
A. cepa	15,000[a]	8	2,000 [92]	0.13	614 [89]	19 [90]	32
T. aestivum	17,000[d]	42	3,600[d]	0.2			
L. longiflorum	19,500[a]	12			2,000 [93]	55 [94]	36

[a]www.rbgkew.org.uk/cval
[b]www.maizegdb.org
[c]http://www.ncbi.nlm.nih.gov
[d]P. Sourdille, pers. com.
[e]L. Anderson, pers. com.
[f]The ratio CO/DSB is calculated by considering that the number of ENs or RAD51/DMC1 foci is equivalent to the number of DSB sites.

tends to prevent another one in that pair' [7]. It remains unknown what mediates interference, but recent data obtained in *Saccharomyces cerevisiae* and *Arabidopsis thaliana* have demonstrated variability in the strength of interference [8, 9]. This suggests that, regardless of the nature of the signal indicating the presence of a CO or pre-CO (physical, molecular or chemical), its propagation along the genetic molecule is not linear. Furthermore, not all COs are affected by interference (see below).

On a finer scale, COs tend to be clustered in small regions of only a few kilobases in size. This has been clearly demonstrated in many eukaryotes (reviewed in [10, 11]). These CO clusters – also known as meiotic hot spots of recombination – are centered around programmed meiotic DNA DSBs, from which meiotic recombination is initiated. Data from yeast, mice and humans have shown that COs are not the only product of DSB repair. Indeed, DSBs may also be repaired as Non Crossover (NCOs), which are also known as gene conversion events when they include a genetic marker. The existence of such NCO events in plants has been demonstrated in maize, at the *bronze* locus [12, 13] and in *Arabidopsis* [3]. Furthermore, cytological data from a large number of species suggests that meiotic DSBs are most repaired as NCOs.

Indeed, cytological markers of DSB sites are present in a 10- to 40-fold excess over CO markers (table 1). Moreover, the number of these markers is increased in mutants affecting prophase progression, suggesting that there is an asynchrony in DSB formation and repair in wild type [14]. Thus the number of DSBs is likely underestimated in wild type. Assuming that all meiotic DSBs are repaired as COs or NCOs, these data suggest that NCO events are much more frequent than COs in meiotic cells.

Meiotic Recombination Mechanisms

Meiotic Recombination Initiation: DSB Formation

DSB formation is catalyzed by Spo11 in budding yeast as in the other eukaryotes studied to date [15]. Spo11 displays similarity to the catalytic subunit (TOP6A) of an archeal type VI topoisomerase [16]. Spo11 is encoded by a single gene in most higher eukaryotes other than plants, which contain several putative Spo11 homologs [17–19]. Furthermore, plant genomes encode homologs of the topoisomerase VI B subunit, which is absolutely necessary for the topoisomerase function in the archaebacteria [20]. In *Arabidopsis*, the disruption of *AtSPO11–1* or *AtSPO11–2* induces a typical asynaptic phenotype (fig. 1A) associated with a dramatic decrease in meiotic recombination, leading to the formation of achiasmatic univalents, which is correlated with an absence of meiotic DSBs [17, 21]. The lack of functional redundancy between the two Spo11 homologs suggests that DSB formation could be catalyzed by a Spo11 heterodimer in plants, whereas it would be a homodimer in the other eukaryotes [22]. Unlike AtSPO11–1 and AtSPO11–2, neither AtSPO11–3 nor AtTOP6B (the topo VIB homolog from *Arabidopsis*) are involved in meiosis, instead they play a major role during somatic development [23–25], suggesting that plants have retained a topoisomerase VI function in addition to the meiotic specialization common to higher eukaryotes observed for Spo11.

In *Saccharomyces cerevisiae*, Spo11 requires nine additional proteins for meiotic DSB formation (fig. 1), but very little is known about the molecular functions of these proteins [15]. Only four of these proteins are conserved throughout the plant kingdom (Rad50, Mre11, Nbs1, Ski8), but none have conserved their function in DSB formation in plants [26–31]. However, forward meiotic mutant screening has led to the identification of AtPRD1, which is required for meiotic DSB formation, as *Atprd1* mutations abolish the DSB repair defects of a large range of meiotic mutants (including *Atrad51* mutant) [32]. AtPRD1 displays sequence similarity to the vertebrate protein Mei1, which is involved in early meiotic recombination [33], suggesting that higher eukaryotes may have mechanisms governing the initiation of meiotic recombination in common.

Based on the phenotype of the DSB-defective mutants in *Arabidopsis* described above, some of the other meiotic genes described in plants may also act at this step of meiotic recombination. This is the case for the *SDS* gene, which encodes a meiosis-specific cyclin-like protein [34].

Early Steps of DSB Repair

DSB Processing. During meiotic cell division in *S. cerevisiae,* the MRX complex (consisting of Mre11, Rad50, and Nbs1/Xrs2) is required for the formation of meiotic DSBs, catalyzed directly by Spo11. The MRX complex is also necessary for DSB processing, as it is required for the release of Spo11 from the DSB [15]. The functions of both MRE11 and RAD50 have been studied in *Arabidopsis*. The corresponding mutants display defect in synapsis and chromosome fragmentation during meiosis; this fragmentation is barely detectable during prophase, but is massive from metaphase I onwards [27, 29]. Large chromatin 'blobs', the nature of which has yet to be characterized, are also visible at metaphase I (fig. 1B). The fragmentation in *Atmre11* has been shown to be AtSPO11–1-dependent [27]. Thus, both AtRAD50 and AtMRE11 are required for DSB processing, but not for DSB formation. AtMRE11 and AtRAD50 have also been shown to interact physically [35]. These data suggest that the function of the MRX complex in meiotic DSB processing is conserved from yeast to *Arabidopsis*, whereas no such conservation is observed for the DSB formation function of this complex. An NBS1/XRS2 homolog has recently been identified in the *Arabidopsis* genome, but its possible function in meiosis has yet to be analyzed [30].

Another protein, in addition to the MRX complex, is required for Spo11 release and DSB processing in budding yeast [36]. This protein, Com1/Sae2, was believed to be fungal specific but homologs have been recently identified in all eukaryotic kingdoms including plants, where it appears to play the same role in Spo11 release [P. Schlogelhofer, pers. comm.].

DSB Repair/Strand Invasion. DNA processing at the site of DSB generates single-stranded tails. These tails are loaded with DNA strand-exchange proteins to form nucleoprotein filaments, which are thought to be involved in active homology searches and strand exchanges [37]. The functions of several proteins involved in this process have been analyzed in *Arabidopsis*.

Rad51 and Dmc1 are both RecA homologs but play unique and different roles during yeast meiotic DSB repair. Both proteins have been identified in *A. thaliana*, and characterization of the corresponding mutants has revealed major differences in their role. *Atrad51* mutants fail to repair meiotic DSBs, as shown by extensive *AtSPO11–1*-dependent chromosome fragmentation during meiosis [38]. In contrast, the chromosomes of *Atdmc1* mutants do not fragment but have no chiasmata; DSBs seem to occur normally in this mutant but are repaired, presumably using the sister chromatid as a template [39, 40]. One function of AtDMC1 may therefore be to prevent DSB repair between sister chromatids, or to favor inter-homolog repair. In contrast, AtRAD51 may initiate homology searches regardless of the target. Recently, ASY1, an axis-associated protein related to the yeast Hop1, has been proposed to play a key role in coordinating the activity of the RecA homologs to create a bias in favor of inter-homolog recombination [41]. Disruption of the two RAD51 homologs present in maize results in milder defects than observed in *Arabidopsis*, suggesting possible complementation of their function by other RecA-related proteins [42].

In addition to *AtRAD51* and *AtDMC1*, the five *RAD51* paralogs identified in vertebrates are also present in the *Arabidopsis* genome [43]. The products of only two of these genes – *AtRAD51C* and *AtXRCC3* – are involved in meiosis. Phenotypic analyses of *Atrad51c* and *Atxrcc3* mutants and two-hybrid assays suggest that these proteins cooperate with AtRAD51 at this step of meiotic recombination [43–47].

Several proteins are thought to assist DMC1/RAD51 in strand invasion: homologs of the breast cancer susceptibility gene *BRCA2* product and of the Mnd1/Hop2 complex were recently identified as key players in meiotic recombination in *Arabidopsis*, probably in cooperation with the recombinases. Indeed, the silencing of the two *AtBRCA2* genes by RNAi, or the mutation of either *AtMND1* or *AHP2*, leads to severe meiotic defects resembling those of the *Atrad51* mutant – chromosome fragmentation without prior chromosome synapsis [40, 48, 49] (fig. 1C). The fragmentation defect in *AtBRCA2* RNAi and the *Atmnd1* mutant is AtSPO11–1-dependent. Lastly, both AtBRCA2 and AtMND1 interact with either AtDMC1 or AtRAD51 [50]. These data suggest that both AtBRCA2 and the AtMND1/AHP2 complex are essential for meiotic recombination, in direct collaboration with AtRAD51 and AtDMC1. In addition, the maize *PHS1* gene, which seems to be plant-specific, is thought to be involved in recruiting the strand invasion machinery [51].

Finally, another group of proteins, the cohesins, which play a major role in sister chromatid cohesion, appear to also be required for meiotic recombination, either in DSB repair [52–54] or for meiotic DSB formation [55].

Later Steps of DSB Repair: The CO Pathways

As discussed above, there is strong evidence to suggest that DSB repair gives rise to at least two different genetic products (COs and NCOs) in plants, as in other eukaryotes. Very little is currently known about the mechanisms by which NCOs are generated, with the exception of the possible involvement of a synthesis-dependent strand annealing pathway [56].

In the CO pathway, it is possible to distinguish class I COs, which are interference-sensitive, from the randomly distributed class II COs (fig. 1). At the two extremes are *C. elegans,* which has only interference-sensitive COs, and *S. pombe,* which has only randomly distributed COs [57]. In *S. cerevisiae*, class I CO formation is dependent on the ZMM proteins (Zip1, Zip2, Zip3, Zip4, Msh4, Msh5 and Mer3) [58] and, to a lesser extent, on Mlh1 and Mlh3. Class II COs require the Mus81 and Mms4 proteins [59].

The Class I CO Pathway. The existence of two CO pathways in plants was first suggested by Copenhaver et al. [60]. This hypothesis is supported by the recent characterization of several *Arabidopsis ZMM* homologs (*AtMSH4* [61], *AtMSH5* [F. C. H. Franklin and R. Mercier, pers. comm.], *AtMER3/RCK* [62, 63], *AtZYP1* [64] and *AtZIP4* [14], as well as *AtMLH3* [65] and *AtMLH1* [66]) and by immunocytological studies of MLH1 protein in tomato [67].

The disruption of these *Arabidopsis ZMM* genes seems to have no effect on early meiotic prophase events, but systematically leads to much lower levels of CO formation [14, 61, 62, 65]. Studies on the residual COs found in a *zmm* mutant background showed no effect of interference on the COs present in *Atmsh4*, *Atmer3* and *Atzip4* [14, 61, 62]. The most affected *Atzmm* mutants retain 15% of the wild-type level of CO, suggesting that at least 15% of COs in *Arabidopsis* are independent of the ZMM pathway. RNAi-mediated depletion of the two AtZYP1 proteins (major components of the transverse filament of the synaptonemal complex) decreases CO formation by 20%, and results in a high level of non homologous associations and multivalent formation [64]. Thus, *Arabidopsis* transverse filament function seems to play a greater role in controlling homologous chromosome recombination than class I CO maturation.

Little is currently known about epistatic relationships between the *Arabidopsis ZMM* genes, except that *AtZIP4* and *AtMSH4* belong to the same pathway [14], and that *Atmsh*5 is epistatic to *Atmer3* [R. Mercier, unpublished data]. Finally, reciprocal immunolocalization of AtMSH4, AtMLH1, and AtMLH3 has shown that AtMSH4 appears earlier than AtMLH3 on chromosomes and that the localization of AtMLH3 depends on AtMSH4, whereas that of AtMSH4 does not depend on AtMLH3. The colocalization of AtMLH1 and AtMLH3 is observed [65]. Thus, all the evidence suggests that AtMSH4 (and probably AtMSH5) act earlier than AtMLH1/3 but in the same pathway together with AtZIP4 and AtMER3. A recent study of MLH1 immunolocalization in tomato pollen mother cells showed that only a subset of strongly interfering LNs are recognized by anti-MLH1 antibodies [67], suggesting that AtMLH1 is probably a marker of class I CO only, in plants.

Based on the phenotype of the *zmm* mutants in *Arabidopsis*, some other described plant meiotic genes may belong to this class. *PTD,* for example, encodes a protein that is conserved in plants and has a C-terminal domain in common with the DNA repair proteins Ercc1/RAD10 and XPF/RAD1 [68] and MPA1, which encodes a metalloprotease of the M1 family (puromycine-sensitive metallopeptidase) [69]. Further characterization is required to confirm the involvement of these proteins in the CO maturation pathway, but these proteins may provide clues to new components of the class I CO maturation process and insight in features specific to plants.

The Non-Interfering Pathway of CO Formation in Plants. Mus81 is a highly conserved endonuclease that acts with Eme1/Mms4 in the formation of class II CO. The rice genome contains a *MUS81* homolog whose meiotic role has not been studied yet [70]. Concerning *Arabidopsis* two putative *MUS81* genes have been described, one of which has been shown to be involved in somatic DNA repair [71, 72], and accounts for 9% of all COs [71]. Nevertheless, as observed in yeast [59, 73], when both interfering and non-interfering pathways are simultaneously disrupted in *Arabidopsis* COs remain [71], suggesting the possible existence of a third mechanism for CO formation. The second *MUS81* putative homolog is thought to be a pseudogene [71, 72] and no putative EME1 homolog has yet been characterized.

Concluding Remarks

Recombination has remained a mystery for more than a century. The last few years have seen a tremendous increase in our understanding of the mechanisms governing meiosis in various organisms, including plants. Let's hope that the next few years will be at least as exciting!

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Meiosis in Cereal Crops: the Grasses are Back

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Abstract

A major goal of breeding programs is to increase and manipulate the genetic diversity of crops. The incorporation of beneficial genes from wild relatives into crops is achieved by producing hybrid plants in which meiotic recombination events occur between the two genomes. Furthering our understanding of meiosis in the cereals could enable the manipulation of homolog pairing and recombination, significantly enhancing the efficiency of breeding programs. The main obstacle to the genetic analysis of meiosis in cereal crops has been the complex organization of most cereal genomes, many of which are polyploid. However, the recent sequencing of the rice genome, the use of insertional mutagenesis and reverse genetics approaches has opened the door for the genetic and genomic analysis of cereal meiosis. The goal of this review is to show how these new resources, as well as the use of three-dimensional microscopy, are rapidly providing insights into the mechanisms that control pairing, recombination and segregation of homologous chromosomes during meiosis in four major cereal crops: wheat, rice, maize and rye. Copyright 2009 © S. Karger AG, Basel

The correct segregation of chromosomes during meiosis is achieved through a series of complex changes at the level of DNA molecules, chromosome structure and nuclear organization. These include: replication of DNA, remodeling and pairing of homologous chromosomes, the assembly of a proteinaceous scaffold between the homologs (the synaptonemal complex, SC), and the formation of crossover recombination events between DNA molecules of paired homologs [1]. Inter-homolog crossovers in combination with sister chromatid cohesion provide the basis of temporary physical links that hold the homologs together until the first meiotic anaphase. Homolog segregation is then followed by the separation of sister chromatids during the second meiotic division; thereby, producing four haploid cells [2].

Elucidating the mechanisms that control this complex 'chromosomal dance' requires the ability to combine different experimental approaches, including: cytology, biochemistry, genetics and genomics. Historically, cereal crops have provided favorable models for cytological studies of meiosis; in fact, the observations made by Creighton and McClintock in 1931 on maize meiotic chromosomes demonstrated the

link between genetic recombination and cytological crossovers [3]. However, until the genome of *Arabidopsis thaliana* was sequenced, plants were lagging behind other model organisms for the study of meiosis due to the lack of genomic resources. More recently, sequencing of the rice genome has brought cereal crops into the genomics era opening new doors for the study of meiosis in this group of plants. Many efforts are now being devoted to increase the genomic resources available in cereal crops including genome-wide sequencing of maize (www.maizesequence.org), *Sorghum* (www.phytozome.net/sorghum) and *Brachypodium* (www.brachypodium.org). Projects are also in place to physically map and sequence the gene space for barley (http://barleygenome.org) and wheat (www.wheatgenome.org). Combining these efforts will facilitate the genetic analysis of meiosis in cereal crops in the imminent future.

Why Should we Study Meiosis in Cereal Crops?

The study of a wide range of organisms has played an important role in our current knowledge of meiosis. Although many meiotic genes are well conserved, the interplay between processes such as pairing, synapsis and recombination shows some important differences between species studied so far. For example, *Arabidopsis* mutants lacking a component of the central region of the SC display formation of crossovers between non-homologous chromosomes, a phenotype that is not observed in available mutants of SC components in any other species [4]. Despite the recent progress made in our understanding of meiosis in *Arabidopsis*, a dicotyledonous plant, our knowledge of meiosis in the monocotyledonous plants remains very limited [5]. This group of flowering plants diverged from the dicots around 200 million years ago and includes the cereals, a group that represents more than 60% of the worldwide agricultural production.

Since its domestication about 10,000 years ago wheat (as well as other crops) has been subjected to a strong breeding selection that created a bottleneck effect on the genetic diversity of today's crops [6]. In the case of wheat it is calculated that only about 15% of the variability present in wild relatives has been captured [7]. One of the main goals of breeding programs is to cross back into cereal crops genes responsible for beneficial traits found in their wild relatives. However, in many instances the crop genome has diverged from its wild relative beyond the point where their chromosomes are able to undergo homologous recombination during meiosis. A solution to this problem has been the use of mutant backgrounds, such as the *Ph1* mutant in wheat, that reduce the fidelity of homolog pairing and synapsis and allow recombination to take place between related, but non-homologous, chromosomes [8]. Being able to understand and manipulate homolog pairing and recombination in wheat and other cereals could have a significant impact on the efficiency of breeding programs.

Table 1. Composition and sizes of cereal crops compared to model organisms

Species	Genome size (Mb)	Ploidy level	% Repetitive DNA
S. cerevisiae	12 [62]	2n = 2x = 32	3.4 [62]
C. elegans	100 [63]	2n = 2x = 12	16.5 [63]
Arabidopsis thaliana	125 [64]	2n = 2x = 10	13 [64]
Oryza sativa	490 [65]	2n = 2x = 24	35 [66]
Zea mays	2,671 [65]	2n = 2x = 20	78 [66]
Hordeum vulgare	5,439 [65]	2n = 2x = 14	76 [66]
Secale cereale	8,110 [65]	2n = 2x = 14	92 [66]
Triticum aestivum	16,979 [65]	2n = 6x = 42	83 [66]

Structure of Cereal Genomes, Implications for Meiosis

Much of the DNA present in many cereal genomes consists of repetitive sequences (table 1), for example retrotransposons account for more than 50% of the maize genome [9]. During meiosis in cereals, homology search mechanisms need to discriminate between sparse unique sequences present in homologous chromosomes and the widely spread repetitive elements. Despite their big differences in size (table 1), comparative genomic analysis shows that cereal genomes can be reconstructed on the basis of the linkage groups contained in the rice genome and the extensive expansion of repetitive sequences [10, 11]. Therefore, cereals can be thought of as a single genetic system with the important implication that the relatively small, and now sequenced, rice genome can be used as a genetic model for other cereals.

Many cereals carry polyploid genomes where each chromosome has a true homolog plus two (or more) closely related homoeologous chromosomes. In polyploid species the presence of multiple copies of each chromosome can disrupt homolog pairing and synapsis rendering the species sterile. To be fertile, polyploid species need to behave as diploids during meiosis and ensure that crossovers are only formed between true homologs. Two main mechanisms are proposed to be responsible for the 'diploidization' of polyploid species. The first one involves the physical differentiation of the homoeologous chromosomes by changes in their structure and/or DNA sequence. This reduces the overall similarity between homoeologous chromosomes, thereby promoting the association of true homologs. Secondly, polyploids carrying homoeologous chromosomes that have not undergone extensive structural differentiation have acquired genetic systems that are responsible for restricting pairing and recombination to take place only between homologous chromosomes. Evidence for these fascinating pairing control systems has been found in wheat, oats, fescues and even some dicotyledonous species such as *Brassica napus*, and cotton [12], but despite this evidence the precise mechanism by which these systems operate remains to be elucidated.

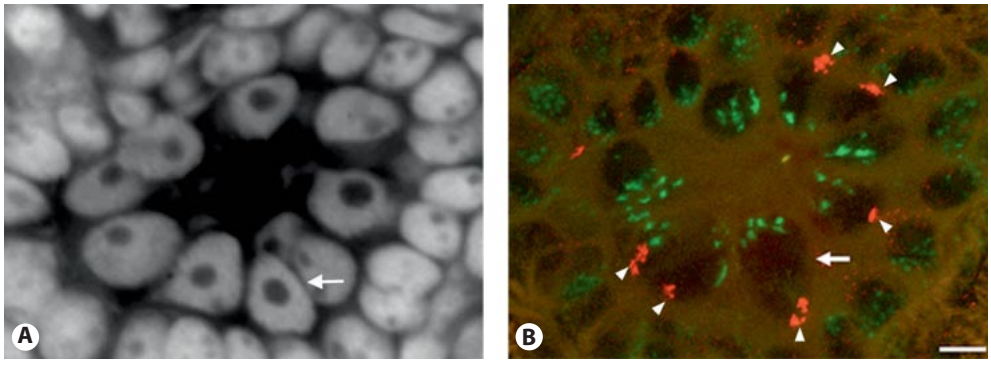

Fig. 1. Synchronous meiosis in tetraploid wheat anthers. **A** Partial projection of an anther section stained with DAPI; a single anther locus is shown, larger nuclei in the inner ring are meiocytes, which are surrounded by tapetal cells. **B** Same locus as in **A**, telomeres shown in red and centromeres in green labeled by FISH. All meiocytes display a telomere bouquet (arrowheads). The same meiocyte is arrowed in **A** and **B**. Bar = 10 μm.

Tools for the Study of Cereal Meiosis

Most cytological studies of meiosis in plants are carried out on the developing meiocytes present inside the anthers. The anthers of cereal crops are much larger that those of *Arabidopsis* and each one contains hundreds of meiocytes developing in synchrony (fig. 1). Furthermore, cereal inflorescences contain multiple flowers that are arranged in a developmental gradient, so anthers from a single inflorescence can provide a complete time course of meiosis.

Cereal meiotic mutants can be identified easily on the basis of the characteristic sterility phenotype: flowers develop normally but grain production is either severely reduced or absent. Although there are many available lines that display this phenotype, elucidating the identity of genes responsible for the sterility phenotype by traditional mapping methods has proven very difficult. This problem has been overcome by insertional mutagenesis, using T-DNA and transposable elements, which allows the identification of the genomic sequences flanking the insertion site [13, 14]. A collection of 50,000 insertion lines in rice has recently been reported to contain 3,825 lines exhibiting the sterility phenotype [15], providing a substantial population of putative meiotic mutants.

Methods for posttranscriptional gene silencing have also been developed for cereal crops. Rice plants can be stably transformed to express 'hairpin' dsRNA of the desired gene and this approach has been successful in knocking down meiotic genes [16]. RNAi technology is expected to be of great importance in studying gene function in polyploid species, such as wheat, where the presence of multigene families means that insertional mutagenesis may not be an effective method to study gene function. Gene targeting technology by homologous recombination has also been developed in rice

[17, 18], and although the technology is currently far from common practice, its development will provide another important tool for the study of meiotic genes.

A microarray expression analysis of meiosis in wheat has identified 1,350 meiotically regulated transcripts [19]. Of these, 30 transcripts displayed at least an eight-fold expression change between different meiotic stages and of them, 16 lack similarities to any database entry, potentially representing wheat-specific meiotic transcripts. Microarray analysis could represent an efficient way of identifying novel meiotic genes in other cereals in which large, and/or, polyploid genomes make the use of more conventional approaches impracticable.

Apart from the species considered in detail below, extensive genomic resources are being developed for other cereals, such as barley, *Sorghum* and *Brachypodium* (see introduction). Both barley and *Sorghum* are crops of great economical importance. The grass *Brachypodium* has the advantage of a small genome (~300 Mbp), short life cycle, and simple growth conditions. Therefore, these plants too will probably become targets of meiotic studies in the near future.

Wheat

Wheat is a hexaploid species that carries three closely related (homoeologous) sets of seven pairs of chromosomes. Elucidating the mechanisms that ensure proper homolog pairing in such a complex genome has been a central goal of wheat researches for decades. Using confocal microscopy on thick anther sections Aragon-Alcaide et al. showed that homologous chromosomes undergo increasing levels of associations before the onset of meiotic prophase, and suggested that these associations started at the centromeres [20]. Later studies confirmed that centromeres associate in pairs before meiosis in wheat [21, 22] as well as in some of its polyploid relatives [23]. Centromere associations probably take place both between homologous and non-homologous centromeres. This is evidenced in wheat/rye hybrid plants that bear a single copy of each chromosome (no homologs are present), and also display association of centromeres in pairs [24]. It is clear that although the pair-wise association of centromeres before meiosis could provide some chromosome sorting, some centromeres are still non-homologously associated at the onset of meiosis, and this needs to be corrected during meiotic prophase.

The onset of meiotic prophase in wheat is defined by the clustering of all the telomeres in a small region of the nuclear envelope. This configuration known as the bouquet stage is observed in many other organisms and is thought to play a role in the homology search process [25]. The first cytological step of bouquet formation is the aggregation of telomeres into small groups that then converge into a single cluster. At the time when telomeres begin to cluster the number of centromeric signals is reduced from ~21 (of the total 42 centromeres present) to ~7 [26]. If each group contains the same number of centromeres, then 6 centromeres would be present per group, raising

the possibility that each group comprises the homoeologous centromeres of all 3 genomes. As the telomere bouquet is fully formed the 7 centromere groups start to appear as tripartite structures that are then fully resolved into 3 separated foci until a total of 21 centromeric signals are observed. At that time homologous chromosomes appear mostly aligned and therefore each centromeric signal must represent a pair of homologous centromeres. Taking advantage of a FISH probe to specifically label rye centromeres, Prieto et al. demonstrated that in wheat/rye hybrids the centromeres also reduce down to 7 groups as telomeres cluster [27]. Each one of these groups contained a single rye centromere, supporting the idea that centromeres move into homoeologous groups at the onset of meiosis. These homoeologous centromere associations may facilitate the pairing process by bringing homologous and homoeologous chromosomes into close proximity, thereby, reducing the overall complexity of the homology search.

A recent study has shown that centromeres also associate in pairs during budding yeast meiosis [28]. Similarly to wheat, centromere associations start as non-homologous and are later transformed to fully homologous associations. Centromere coupling may facilitate homolog pairing by holding homologs together while homology is assessed by other contributing mechanisms such as recombination and the bouquet formation. Although the mechanisms controlling centromere associations in wheat are not known, it appears that centromeres may play a role in homolog pairing in both yeast and wheat.

During early meiotic prophase wheat chromosomes undergo a striking remodeling in which they change from their rod-like appearance before meiosis to a much more stretched out and string-like appearance [21, 22, 27, 29]. Observation of a wheat line carrying a fragment of a rye chromosome (substituting 15% of the equivalent wheat chromosome arm) showed that before entering meiosis the rye segments appear as two small signals, but that at early stages of bouquet formation these are greatly stretched to about 5 times their previous length [27] (fig. 2). Following this stretching, the rye fragments start to associate from the end closer to the telomere, suggesting that homologous chromosome arms are being paired from their telomere moving towards their centromeres. In contrast to the synchronous stretching of the rye fragments observed in wild type wheat, *Ph1* mutant plants displayed asynchronous stretching of the rye fragments in most meiocytes at the bouquet stage. Surprisingly, in the absence of homologous chromosomes (in wheat-rye hybrid plants) the rye subtelomeric heterochromatic knobs did not display a conformational change during the bouquet stage. This suggests that the conformational change is triggered by the interaction of subtelomeric regions of the homologs during the bouquet stage, and that this conformational change in turn allows the intimate pairing of the homologs. In *Ph1* mutants the conformational change can be triggered when the subtelomeric regions of non-homologous chromosomes interact and this results in non-homologous chromosome pairing.

Several studies provide direct evidence for the importance of the bouquet in homolog pairing. Colchicine treatment of wheat meiocytes disrupts the telomere

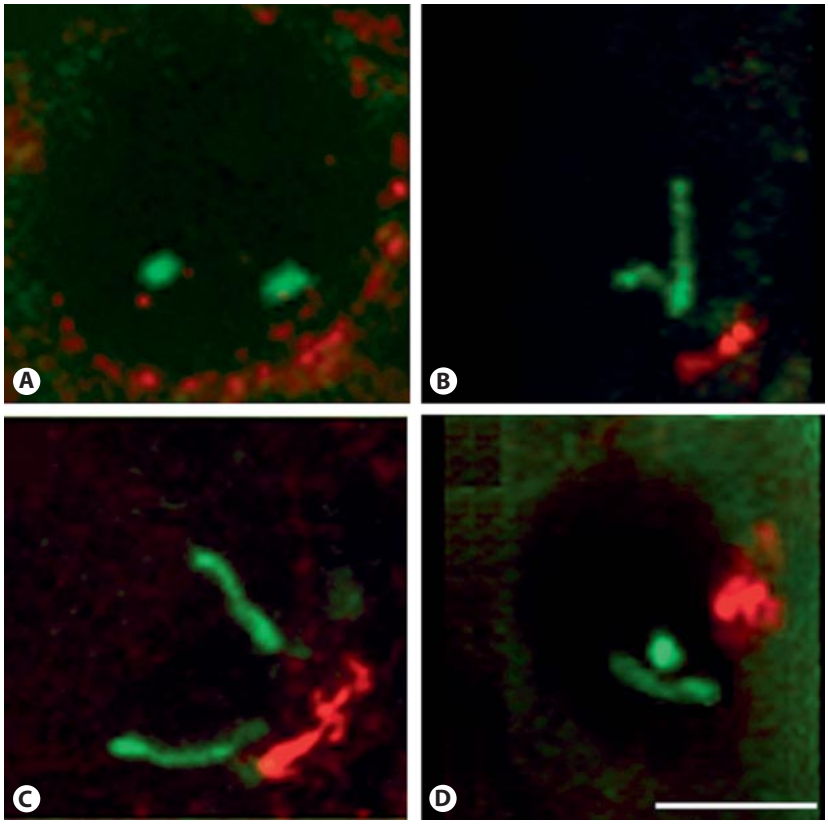

Fig. 2. Homolog pairing and chromosome remodeling in wheat. Partial projections of individual meiocytes labeled by FISH to visualize the telomeres (red) and a rye segment present on wheat chromosome 1D (green) at different stages. **A** Pre-bouquet, telomeres are still not clustered and the rye segments appear as two round signals. **B** Early bouquet, the telomeres have formed a single cluster and the rye segments appear greatly stretched. **C** Mid bouquet, the rye segments start associating from the end closer to the telomere cluster. **D** Bouquet stage in the *Ph1b* mutant, the telomeres have formed a single cluster but only one rye segment has been remodeled while the other one remains in the premeiotic conformation. Bar = 10 μm. Original images provided by Graham Moore.

bouquet and the subsequent intimate alignment of the homologs without disrupting centromeric associations [29]. Simultaneous pairing analysis of distal regions and centromeres demonstrates that meiotic alignment of the homologs starts at the telomeres and then progresses towards the centromere, suggesting that homologous centromere pairing at zygotene is in fact driven by synapsis [30]. Finally, in oat lines carrying a pair of maize homologous chromosomes, alignment of the two maize chromosomes coincided with the bouquet stage, when the maize chromosomes appear as stretched fiber-like structures [31].

The *Ph1* locus in wheat appears to affect the specificity of the centromere associations [24, 27], the timing of chromosome remodeling [22, 27] and homolog alignment

[22, 27]. One of the most intriguing questions in cereal meiosis is: what are the molecular mechanisms by which *Ph1* influences all these processes? The *Ph1* locus is defined by a series of deletions on chromosome 5B, and *Ph1* activity is not detected on any of the chromosomes homoeologous to wheat 5B. These observations, as well as the fact that EMS treatment has failed to yield *Ph1* alleles, suggest that the *Ph1* locus arose by a structural change specific to chromosome 5B. Detailed analysis of a 2.5 Mb region containing *Ph1* revealed the presence of a subtelomeric heterochromatin fragment inserted into a cluster of *cdc2* related genes that is specific to chromosome 5B [32]. This has raised the intriguing possibility that this complex genetic region, rather than an individual gene, may be responsible for *Ph1* activity.

Rice

Rice is a diploid species that carries 12 pairs of chromosomes and its genome is the smallest of the cultivated cereals. Three-dimensional analysis of centromeres and telomeres in premeiotic rice anthers has shown that both the centromeres and the telomeres are found associated in pairs before the onset of meiosis, but not in root nuclei [33]. FISH analysis suggests that most of these premeiotic centromere and telomere associations may take place between homologous chromosomes. This is in contrast with the situation in *Arabidopsis* and maize in which no association of the homologs is observed before the onset of meiosis [34, 35].

The sequencing of the rice genome combined with insertional mutagenesis is driving the genetic analysis of rice meiosis. The *MSP1* gene, which encodes a receptor-like protein kinase, was isolated based on the sterility phenotype of a *Tos17* insertion mutant [36]. Male meiocytes in *msp1* mutants arrest at various stages of meiotic prophase, which is surprising since meiotic arrest and/or cell death is not a common phenotype in plant meiotic mutants [5]. The male meiotic arrest in *msp1* mutants is explained on the basis that during anther development more cells develop as pollen mother cells than in wild type plants, depleting the population of cells that will normally develop as tapetal cells and nurse the proliferating meiocytes. Although female meiocytes do not arrest in *msp1* mutants, the flowers contained several megaspores (instead of one). Therefore, *MSP1* appears to restrict the numbers of cells entering male and female sporogenesis during floral development.

The same genetic screen was successful in the isolation of pairing mutants. *pair2* mutant plants display unsynapsed chromosomes at pachytene and 24 univalents at diakinesis [37, 38]. The *PAIR2* gene encodes an ortholog of the *Arabidopsis* axial element component *ASY1,* which is needed for homolog pairing [39]. Labeling of pollen mother cells with pulses of BrdU showed that *PAIR2* expression starts following premeiotic DNA replication and disappears by pollen maturation. PAIR2 starts associating with the axial element of chromosomes at the onset of meiosis and by the end of leptotene the PAIR2 signal extends over the whole length of unsynapsed chromosomes.

As chromosomes synapsed the PAIR2 signal decreased, suggesting that PAIR2 is removed from chromosomes as synapsis is completed. This staining pattern is reminiscent of that of Hop1 in yeast but is different from ASY1 staining in *Arabidopsis* and rye, where the protein persists on the chromosomal axes of fully synapsed homologs [40–42].

PAIR1 is another gene needed for synapsis in rice that was isolated by insertional mutagenesis. *PAIR1* encodes a coiled-coil protein with homology only to an uncharacterized protein in *Arabidopsis* [43]. Although homolog pairing fails in *pair1* mutants, chromosome morphology appeared largely normal at leptotene and zygotene. Chromosomes even clustered to one side of the nucleolus forming a characteristic synizetic knot, as they do during zygotene in wild type plants. However, in *pair1* mutants the knot is not released at pachytene and chromosomes remained clustered until diakinesis. By then chromosomes were often connected by chromatin bridges but no chromosomal fragments were observed, suggesting that DNA double strand breaks (DSBs) were either not formed or formed but repaired in the absence of synapsis. The authors favor a role of *PAIR1* during presynaptic homolog alignment before loading of the DSB initiation machinery, which would also be consistent with the timing of *PAIR1* expression.

The rice genome contains four *RAD21*-like genes, *RAD21* and its meiotic version *REC8* play essential roles in sister chromatid cohesion [2]. RNAi experiments in rice have shown that *RAD21–3* appears to be a *RAD21* ortholog and is needed for pollen development [44], while *RAD21–4* is a *REC8* ortholog required for meiosis [45]. Knockdown of *RAD21–4* resulted in abnormal condensation of chromosomes during meiosis and the presence of chromosome fragments at diakinesis. FISH in pachytene nuclei demonstrated a partial failure of homolog pairing as well as some sister chromatid separation on chromosome arms. In contrast, FISH with a probe for centromeric regions revealed no premature separation of sister centromeres in *RAD21–4* RNAi plants at pachytene, although the results from this study need to be taken cautiously since western blot analysis revealed that the RAD21–4 protein levels were only reduced by 50%. Interestingly, in *Arabidopsis syn1*(*rec8*) mutants cohesion is also lost at chromosome arms during meiotic prophase, but retained at centromeres [46]. These observations raise the intriguing possibility that the *REC8* orthologs of *Arabidopsis* and rice may play no role in centromeric cohesion during meiosis, or that there is functional redundancy between *REC8* and one, or more, of the other three *RAD21*-like genes present in plants.

RNAi has also been used to study the role of rice *DMC1* [16]. *DMC1* knockdown plants show a partial pairing defect at pachytene (50% of wild type levels), asynchronous meiosis and a mixture of univalents and bivalents at diakinesis. Biochemical characterization of rice DMC1 in vitro shows that the enzyme exhibits many hallmarks typical of recombinases including: binding to double- and single-stranded DNA, renaturation of complementary strands and strand exchange function [47].

Maize

Maize is an ancient tetraploid species, but the extensive rearrangements experienced by its two ancestral genomes mean that it behaves cytologically as a true diploid. The exceptional cytology offered by maize chromosomes allowed this plant to be the first cereal species studied by 3-dimensional microscopy [48]. This early study showed that homolog pairing is preceded by a dramatic structural reorganization of the chromosomes and suggested that intimate homolog alignment started at the telomeres. A later study showed that telomeres start meiosis dispersed throughout the nuclear volume but that at the end of leptotene they move to the nuclear envelope and form a single cluster (the bouquet stage), which is then released during pachytene when the homologs are fully synapsed [49]. Therefore, telomere clustering appeared to precede homolog synapsis. The coincident timing of initial homolog pairing and the telomere bouquet was confirmed by observations in an oat line carrying a pair of maize homologs [31].

In contrast to wheat and rice, maize centromeres are not associated in pairs before zygotene [35]. However, during premeiotic interphase and leptotene, nuclei of maize meiocytes display a polarized organization in which all the centromeres are present in the same nuclear hemisphere. This suggests that centromeres play an important role in the nuclear organization of early meiocytes. The observation of maize lines carrying telocentric and ring chromosomes (both of which have a centromere immediately adjacent to a telomere) demonstrated that at late leptotene nuclear organization switches dramatically from a centromere-led polarization to a telomere-led organization [35].

None of the genes that control the formation of the telomere bouquet have been cloned in maize, but there are several mutants in which primary defects appear to occur at the bouquet stage. In the *pam1* mutant the telomeres attach to the nuclear envelope and even form some mini clusters, but they fail to converge into a single cluster [50]. Events preceding the bouquet, such as centromere polarization are not affected in *pam1* mutants, while later events such as pairing and synapsis are disrupted. In *dsy1* mutants only partial bouquets are formed, with some telomeres never migrating to the bouquet and improper homolog pairing and synapsis are observed at pachytene [51]. The analysis of the *pam1* and *dsy1* mutants suggests a functional link between the bouquet and homolog pairing and synapsis. Finally, in *dy* mutants the bouquet is successfully formed, but by mid-pachytene the telomeres are prematurely released from the nuclear envelope and move inside the nucleus [51]. *dy* mutants show reduced recombination rates, so it is possible that there is interplay between meiotic recombination and maintenance of telomere attachment to the nuclear envelope.

During meiotic recombination DSBs are formed and processed to expose single-stranded DNA, which is then bound by the recombination protein RAD51 and can be used as a template to check for complementary DNA sequences. In wild type maize, individual RAD51 foci appear at leptotene and peak at mid-zygotene when they average almost 600 foci per nucleus [52]. At this time homologous chromosomes are actively pairing and most RAD51 foci are located on unpaired chromosomes. Paired

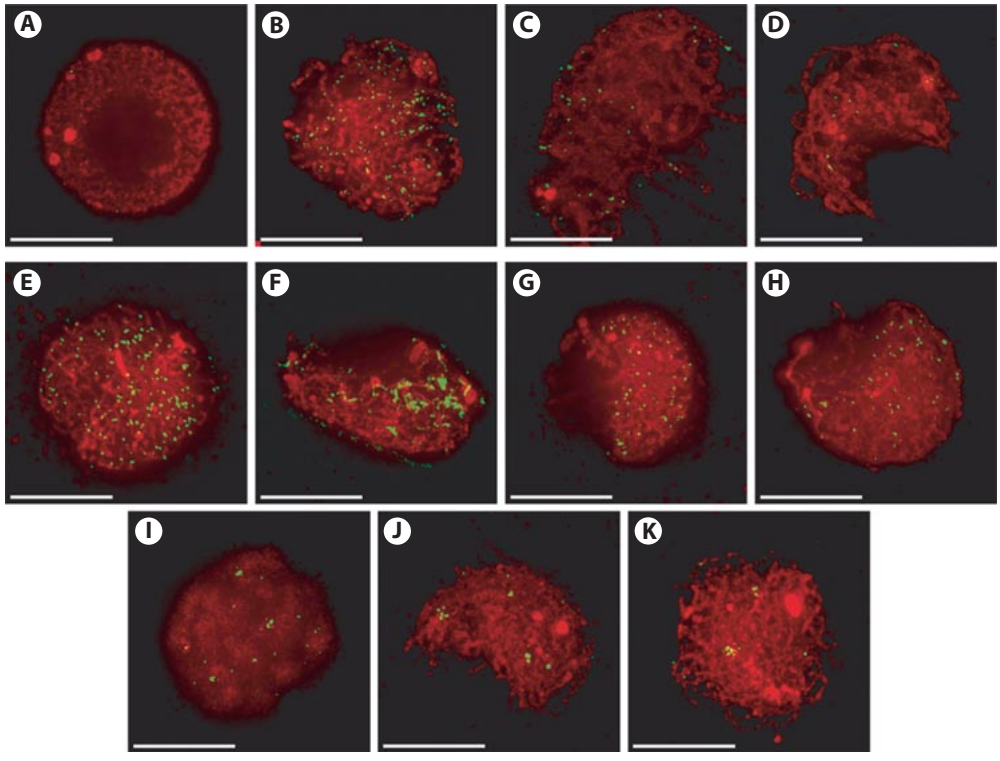

Fig. 3. Distribution of RAD51 foci in wild type and mutant maize meiocytes. **A**–**D** RAD51 immunostaining in wild type maize meiocytes in leptotene (**A**), zygotene (**B**), late zygotene (**C**), and pachytene (**D**). **E**–**K** RAD51 staining in zygotene nuclei of maize meiotic mutants, *dsy1–1* (**E**), *as1* (**F**), *mtm99–25* (**G**), *dsy9901* (**H**), *afd1* (**I**), *segII-513* (**J**), and *dsyCS* (**K**). DAPI-stained chromatin is in red, RAD51 is in green. Bar = 10 μm. The RAD51 staining on the outside of the nuclear envelope was detected in all stages of prophase I in most mutants as well as in the wild type controls and was previously reported in wild type maize meiocytes [52]. It likely represents RAD51 protein being imported into the nucleus or a contaminating non-specific staining of the HsRAD51 antibody preparation [52]. Original images provided by Wojtek Pawlowski and Zac Cande (Image Copyright holders ASPB).

RAD51 foci appear during zygotene and by late zygotene, when almost all chromosomes are fully paired, the overall number of RAD51 foci is greatly reduced. By pachytene only an average of 15 foci were observed, which is only slightly lower than the 19 chiasmata observed (on average) per nucleus [53]. At their peak, RAD51 foci exceed the number of crossovers by 25 times, raising the possibility that RAD51 may play a role in the homology search that ensures proper pairing. In support of this idea, several meiotic mutants that display pairing defects also show altered patterns of RAD51 foci (fig. 3) [54, 55]. Analysis of plants carrying mutations in the two copies of the maize *RAD51* genes (the most similar to *Arabidopsis* and yeast *RAD51*) has confirmed a role of RAD51 in the homology search [53]. These plants not only display synapsis between non-homologous chromosomes, but they also display multivalents

as well as heteromorphic bivalents at diakinesis, demonstrating recombination between non-homologous chromosomes. This phenotype is not observed in any other maize meiotic mutant or in other *rad51* mutants studied in other organisms, including *Arabidopsis*. Thus, RAD51 plays a key role in the fidelity of homolog pairing and recombination during maize meiosis.

The *PHS1* gene, first identified in maize, appears to be a key coordinator of different meiotic events including pairing synapsis and recombination [56]. *phs1* mutants display wild type levels of synapsis, but almost all tracks of SC are formed between non-homologous chromosomes. RAD51 foci are almost completely absent, although DSBs appear to be formed. This suggests that *PHS1* may play a role in loading of recombination proteins (including RAD51) following DSBs formation, and that this is a key step in the coordination of pairing and synapsis.

Analysis of an allelic series of the *AFD1* gene, a maize *REC8* homolog, has demonstrated an effect of the structure of meiotic chromosomes on later meiotic events [57]. All four *afd1* mutant alleles analyzed were able to initiate loading of the axial element component ASY1, but the elongation of axial elements that takes place at leptotene was severely impeded in the strong alleles, which in turn disrupted bouquet formation. However, the bouquet was formed in some nuclei of the weaker *afd1* allele, which displayed up to 50% of the total length of axial element observed in wild type. Thus, axial element elongation appears to be needed for bouquet formation. By pachytene, all alleles showed altered chromatin structure, pairing failure, greatly reduced levels of RAD51 foci and aggregations of lateral elements; demonstrating that all these processes require intact meiotic chromosome axes.

afd1 (*rec8*) mutants exhibit complete loss of sister chromatid cohesion at anaphase I, suggesting that *AFD1* is required for cohesion of both chromosome arms and centromeres. A homolog of Shugoshin (*SGO1*) ensures maintenance of centromeric cohesion before prophase II and correct segregation of chromatids during meiosis II in maize [58]. SGO1 starts localizing to maize centromeres during leptotene and its location to meiotic centromeres is dependent on *AFD1*. Thus, maize *AFD1* may play a more prevalent role in ensuring meiotic centromere cohesion than the *REC8* orthologs of rice and *Arabidopsis*.

Rye

Rye is a close relative of wheat that carries a diploid genome with seven pairs of chromosomes and offers excellent cytology. Before entering meiosis the nucleus of rye meiocytes is clearly polarized, with telomeres and centromeres occupying opposite poles. In contrast with wheat, centromeres are not associated in pairs at this time [59]. The onset of meiosis coincides with the clustering of telomeres on the nuclear envelope; thus, the timing of the bouquet is similar to wheat and earlier than that reported for maize and other organisms. Anther culture of rye meiocytes has demonstrated

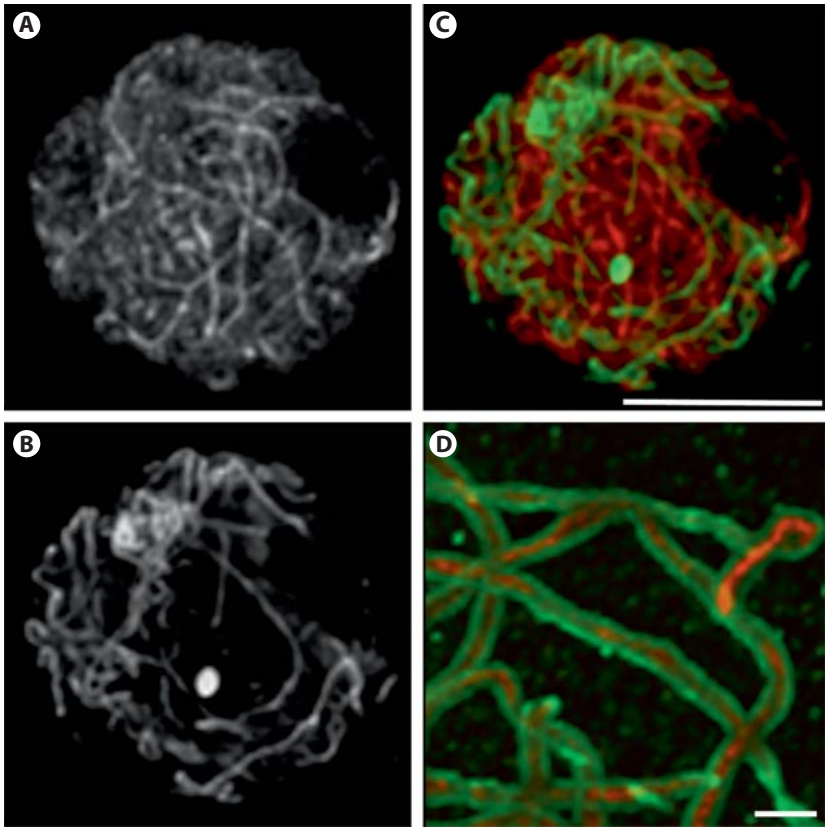

Fig. 4. Synaptonemal complex loading in rye. **A** Partial projection of a zygotene nucleus labeled with anti-ASY1 antibodies. **B** Same nucleus as **A**, labeled with anti-ZYP1 antibodies. **C** Overlay of **A** and **B**, ASY1 (red), ZYP1 (green). Notice that most linear stretches of both proteins do not overlap. **D** Detail of a pachytene nucleus labeled with ASY1 (green) and ZYP1 (red). Bar = 10 μm for **A**–**C** and 1 μm for **D**. Original images provided by Glyn Jenkins.

that the whole cell is polarized de novo coinciding with the formation of the bouquet, suggesting that the single telomere cluster may be positioned relative to the overall polarization of the cell at that time [60]. By zygotene, the bouquet is loosened and the centromeres appear as stretched structures, which are now often found in pairs, suggesting homolog alignment.

SC assembly has been studied in rye by using antibodies against the *Arabidopsis* SC components ASY1 (lateral element) and ZYP1 (central region). In *Arabidopsis*, ZYP1 is initially loaded as individual foci on chromosome axes containing ASY1, and long ZYP1 stretches are observed coinciding with synapsis of the homologs [4]. At leptotene in rye, anti-ASY1 staining demonstrates the extensive presence of ASY1 tracks, while ZYP1 forms short linear structures [42]. Unexpectedly, these early ZYP1 linear structures do not overlap with the linear structures formed by ASY1 (fig. 4) [42]. Assuming that in rye both ASY1 and ZYP1 are loaded on chromosome axes, the lack

of overlap between ASY1 and ZYP1 implies that ZYP1 associates initially on unpaired chromosomal regions. By late zygotene synapsis is virtually completed and the two proteins largely colocalize. Finally, by pachytene the tripartite nature of the SC can be clearly observed as two parallel ASY1 staining tracks sandwiching a single track of anti-ZYP1 signal (fig. 4) [42]. At that time both centromeres and telomeres are observed in pairs suggesting that homologs are fully synapsed.

Large collections of meiotic mutants are available in rye [61] and although none have been cloned so far, several have been studied recently. In *sy1* mutants most nuclei fail to form a tight bouquet, instead several small clusters are formed that persist until pachytene [59]. Centromeres are prematurely dispersed from their grouping in one pole of the nucleus and at pachytene no mature SC structures are observed. By diakinesis chromosomes are observed as univalents. In *sy9* mutants the bouquet is normally formed but centromeric regions become distended, and no mature SC is observed. By diakinesis, very few bivalents are observed. A third mutant, *sy10,* displays non-homologous synapsis as well as unsynapsed regions at pachytene. But perhaps the most striking phenotype is the apparent presence of chiasmata between non-homologous chromosomes at diakinesis [42]. Although there is no direct proof of actual recombination between non-homologous chromosomes, these chiasmata appear cytologically indistinguishable from those seen between homologous chromosomes. Interestingly, evidence for chiasmata between non-homologous chromosomes has so far been only found in different meiotic mutants of plants: wheat *(ph1), Arabidopsis (zyp1)*, maize *(rad51)* and rye *(sy10)*.

Concluding Remarks

The initial analysis of meiosis in the cereals has uncovered novel meiotic genes as well as some unexpected phenotypes in mutants of conserved genes. It is possible that this reflects the existence of cereal-specific meiosis mechanisms that have evolved to ensure correct pairing, recombination and segregation of homologous chromosomes in the context of the large and complex cereal genomes. The large repertory of techniques currently available, combined with the increasing genomic resources, should provide the keys to understand and manipulate the mechanisms that control pairing and recombination in these plants.

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Homologue Pairing, Recombination and Segregation in *Caenorhabditis elegans*

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Abstract

Meiosis in the free-living, hermaphroditic nematode *Caenorhabditis elegans* is marked by the same highly conserved features observed in other sexually reproducing systems. Accurate chromosome segregation at the meiotic divisions depends on earlier landmark events of meiotic prophase, including the pairing of homologous chromosomes, synapsis between them, and the formation of crossovers. Dissection of these processes has revealed a unique simplification of meiotic mechanisms that impact the interpretation of meiotic chromosome behaviour in more complex systems. Chromosome sites required for chromosome pairing are consolidated to one end of each chromosome, the many sites of recombination initiation are resolved into a single crossover for each chromosome pair, and the diffuse (holocentric) kinetic activity that extends along the length of the mitotic chromosomes is reduced to a single end of each meiotic chromosome. Consequently, studies from the nematode have illuminated and challenged long-standing concepts of homologue pairing mechanisms, crossover interference, and kinetochore structure. Because chromosome pairing, synapsis, and recombination can proceed independently of one another, *C. elegans* has provided a simplified system for studying these processes and the mechanisms mediating their coordination during meiosis. This review covers the major features of *C. elegans* meiosis with emphasis on its contributions to understanding essential meiotic processes.
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Meiotic chromosome pairing consists of at least three successive steps that pair homologues and bring them into increasing proximity: a long range interaction which facilitates homologue recognition; the alignment of identical chromosome pairs along their lengths at a distance (also known as homologue juxtaposition or presynaptic alignment); and an intimate association of aligned homologues at a distance of 100 nm through synapsis [1]. These events are combined with dramatic changes in chromosome morphology; first, the assembly of a proteinaceous axial element between newly replicated sister chromatids, and second, the polymerization of transverse filament proteins between the axes of homologous chromosomes, giving rise to the central element of the tripartite proteinaceous structure that defines synapsis, the synaptonemal complex (SC) [2].

While studies from plant, animal, and fungal model systems collectively suggest that the process of homologue recognition is independent of the recombination pathway, different organisms show different dependencies on meiotic double-strand break (DSB) formation and recombination initiation in achieving presynaptic alignment and synapsis [1]. At one end of the spectrum, in mammals, plants and fungi, wild-type levels of DSB formation are required to form recombination-based connections between homologous chromosome axes to achieve presynaptic alignment [1], followed by the initiation of synapsis at the sites of a subset of axial associations destined to become crossovers [3]. Consequently, in these organisms the recombination pathway is functionally interdigitated with presynaptic alignment and synapsis; meiotic DSB formation and recombination are required for presynaptic alignment and the initiation of synapsis, and SC formation is required for the maturation of axial associations into crossovers. At the other end of the spectrum, presynaptic alignment and synapsis proceed in the absence of DSBs and recombination in *Drosophila* [4] and in *C. elegans* [5], culminating in homologously synapsed chromosome pairs. The fact that SC formation and recombination initiation can occur independently of one another in the nematode has made possible their analysis without the added complication that defects in one process impact the other. *C. elegans* is genetically tractable and meiotic mutants can be isolated on the basis of the spontaneous segregation of males (5A; XO) amongst the self-progeny of hermaphrodites (5A; XX) as a consequence of X chromosome nondisjunction [6]. Furthermore, the development of cytological tools and meiotic markers for immunolocalisation, coupled with the spatio-temporal organisation of the germ line has transformed the nematode into a cytologically accessible model system for investigations into meiosis (fig. 1).

Mechanisms of Homologue Alignment and Stabilisation by Synapsis

Early work on meiotic chromosome behaviour in the nematode collectively revealed a startling asymmetry within *C. elegans* chromosomes with respect to meiotic behaviour [7]. Free duplications spanning different ends of a chromosome were found to have asymmetric properties with respect to their abilities to cross over with an intact homologue; unattached duplications of the right portion of chromosome I, for example, could undergo crossing over while duplications of the right portion could not [8]. The behaviour of translocations mirrored the observations made with duplications; in individuals heterozygous for a reciprocal translocation, only one portion of the translocation was competent to recombine and segregate from its homologue [9–11]. EM analysis of translocation heterozygotes revealed fully synapsed chromosome pairs [12], indicating that the crossover suppressed regions were engaged in nonhomologous synapsis. These results were ultimately synthesized into the model that information allowing homologous 'pairing' and genetic exchange was localized to one

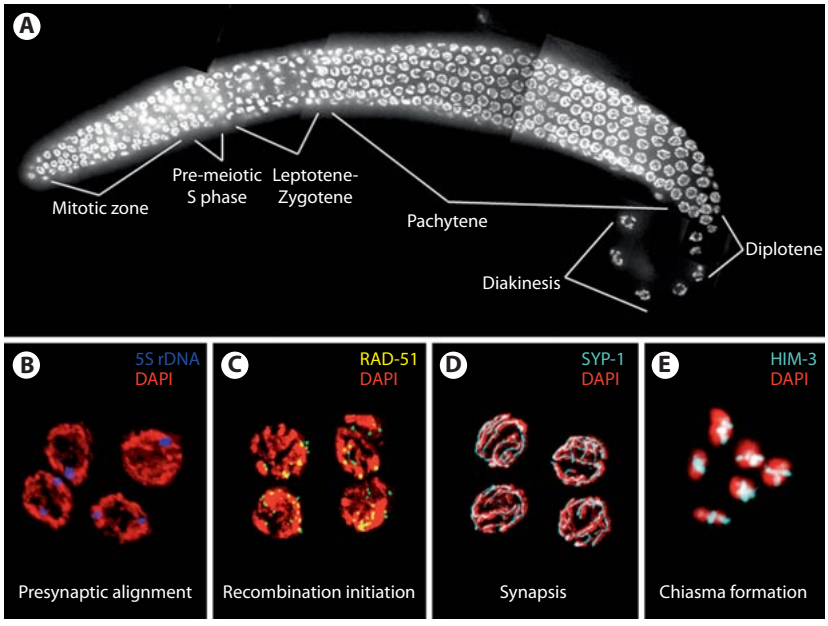

Fig. 1. Spatio-temporal organization of the transparent *C. elegans* germline facilitates visualization of the major events of meiotic prophase. **A** Low magnification image of a DAPI-stained hermaphrodite germline showing prophase progression from left to right. After premeiotic DNA synthesis, nuclei enter the transition zone, corresponding to the leptotene-zygotene; nuclei at this stage adopt a crescent shaped appearance stage as a consequence of spatial reorganization of the nucleus in which chromosomes are clustered to one side. This zone is followed by the pachytene region in which chromosomes attain full synapsis and by nuclei in diplotene in which the chromosomes desynapse and are finally resolved into a condensed and shortened bivalent at diakinesis, the final stage before fertilization. **B** Fluorescence in situ hybridization (FISH) of chromosomes from leptotene-zygotene nuclei undergoing presynaptic alignment; in some nuclei, the 5S rDNA locus on chromosome V is aligned (one signal) while in others alignment has not yet been attained (two signals). Immunofluorescence micrographs of (**C**) early pachytene stage nuclei showing RAD-51-marked early recombination intermediates, (**D**) pachytene nuclei showing synapsed chromosomes in which the central region component SYP-1 localizes between aligned homologues, and (**E**) a diakinesis stage oocyte showing desynapsed chromosomes in which the axis component HIM-3 delineates the chiasmata holding the bivalent together.

end of each chromosome [10], called the Homologue Recognition Region (HRR) [13] or the Pairing Centre (PC) [14].

In *C. elegans*, presynaptic alignment is genetically separable from synapsis. Mutants lacking central region components of the SC achieve presynaptic alignment of homologous chromosomes in the absence of the SC during early prophase, but this pairing is lost at later stages, demonstrating that the maintenance of presynaptic alignment requires stabilization through synapsis [15–17]. During the alignment process, the PC end of each chromosome is the first to pair, suggesting these regions may also contain features required for initial homologue recognition and/or for stimulating localized transient stabilization of homologous contacts. Detailed investigations of

X-chromosome PC function have been possible because the aneuploid progeny arising from X chromosome nondisjunction events are largely viable in *C. elegans*. The X chromosome PC activity is localized to the left end of the chromosome [10] and deficiencies at this end result in high levels of X chromosome missegregation, consistent with a loss or impairment of PC function [14]. Analysis of the effects of these deficiencies on chromosome pairing and synapsis revealed a dual role for the PC region of the X chromosome [18]. First, the PC end of the chromosome is required to establish or maintain the transient pairing observed at this end in the absence of synapsis; since this pairing only occurs in the presence of two intact PCs, the PC may function in both homologue recognition and subsequent presynaptic alignment. Consequently, presynaptic alignment in the nematode may be preferentially initiated at few sites, corresponding to the PCs, while in other systems several such regions are required or the activity is dispersed. Second, the PC end of the chromosome is required for synapsis and possesses an activity that strongly stimulates synapsis, even in PC end deletion heterozygous situations. However, fully synapsed X chromosomes are still observed in 10% of the nuclei of PC region deletion homozygotes and even more attain presynaptic alignment [18], suggesting that either the deletion does not remove all of the pairing and synapsis promoting activity, or these functions require additional chromosomal features outside of the deletion. The latter is supported with the identification of secondary pairing sites along autosomes that may be capable of promoting localized alignment or synapsis [13, 19]. However, sequencing of the breakpoint of one of the PC end deletions revealed that it was capped with sequences from the middle of chromosome V, followed by de novo telomere addition, raising the possibility that the addition of such sequences may affect the meiotic behaviour of the deficiency [20]. Nevertheless, the evidence collectively indicates that *C. elegans* has consolidated chromosomal features required for presynaptic alignment and its stabilization by synapsis to a localized region at the end of the X chromosome, thereby ensuring spatial coordination of the two processes. While the autosomal PC ends have a similar function in presynaptic alignment, their contribution to synapsis initiation is not yet known.

How does the PC work? The *C. elegans* genome encodes a family of four related C2H2 zinc finger proteins, each of which is required for the alignment and synapsis of one or two homologue pairs [21, 22]. Localisation of the X-chromosome specific protein, HIM-8, and its autosomal paralogues (ZIM-1, 2, and 3) revealed that each of the proteins associates with the PC region of the chromosome whose pairing they mediate. The specific localisation of HIM-8 and the ZIMs to PC ends of the chromosomes raises the prospect that these chromosomal regions contain sequences capable of recruiting these proteins. In fact, six repetitive sequence elements have been identified that show a compelling asymmetric and largely chromosome-specific distribution that parallels the known locations of PCs [23]. Although it remains to be seen if these repeats are directly bound by members of the HIM-8 family, the possibility that HIM-8 and the ZIMs have different binding affinities for each of the elements would

explain their predominant localization to one or two PC chromosome ends, although it cannot explain homology recognition per se since two of the ZIMs mediate the pairing of more than one chromosome pair. Moreover, the HIM-8 and ZIM/PC complex associates with the nuclear envelope (NE) throughout early prophase when pairing and synapsis are occurring, raising the prospect that a telomere-NE interaction is required for homologue pairing. In many organisms, the meiotic clustering of telomeric sequences to a single area of the nuclear envelope – referred to as the chromosomal bouquet – is intimately associated with the process of homologue pairing [24]. Although no telomere clustering has yet been observed in *C. elegans* [25], meiotic chromosomes asymmetrically cluster to one side of the nucleus at the stage when homologue pairing and synapsis are taking place. Furthermore, chromosomes fail to cluster in mutants defective in presynaptic alignment, suggesting a mechanistic link between the two processes [26–28]. Consequently, the interaction of PCs (or telomeres in other systems) with the nuclear envelope may represent a conserved physical constraint required for successful homologue interactions; however, the tethering of chromosome ends alone is not sufficient for chromosome pairing [21]. A direct involvement of the nuclear envelope in chromosome pairing has recently been revealed by the analysis of a meiosis-specific allele of the SUN-1 inner nuclear membrane protein [28]. In these mutants, SUN-1 localizes normally and HIM-8 localization and tethering of the PCs to the nuclear envelope is intact, however nuclei are defective in chromosome clustering in early prophase and chromosomes fail to achieve presynaptic alignment. During embryonic cell divisions, SUN-1 targets ZYG-12 to the outer nuclear membrane to mediate nuclear attachment of the centrosome [29]; however during meiotic prophase SUN-1 is required for the formation of centrosome-independent aggregates of ZYG-12 that colocalise with the HIM-8/PC complex. Since ZYG-12 is also required to localise dynein to the nuclear envelope, a tempting scenario is that SUN-1-mediated aggregation of ZYG-12 at sites of PC tethering to the nuclear envelope results in dynein-dependent microtubule forces that are required to position chromosomes during presynaptic alignment and/or to facilitate homologue recognition. A similar requirement for SUN1 in bouquet formation and meiotic chromosome pairing has been observed in mice [30], suggesting a conserved role for nuclear envelope-driven events in these processes and the presence of a zinc-finger domain in the mouse SUN1 homologue [31] raises the possibility that it reduces to a single protein the functions performed by *C. elegans* SUN-1, HIM-8 and its paralogues – a link between the nuclear envelope and meiotic chromosome ends. Whether this type of PC end – nuclear envelope interaction observed by immunofluorescence represents a true telomere attachment of the type observed by EM is not known. In fact, serial EM reconstructions of pachytene nuclei demonstrated that like in other nematodes, while only one end of the chromosome attaches to the nuclear envelope, both ends have the ability to do so [25], although it is possible that chromosome end-nuclear envelope interactions change between leptotene/zygotene and pachytene.

Synaptonemal Complex Structure and Organisation

EM analysis of the SC of *C. elegans* revealed the same conserved tripartite organization observed in other systems; two chromosome axes (known as lateral elements in the context of the SC) separated by a striated central element [25]. In addition to stabilising presynaptic alignment, the SC is also essential for the maturation of recombination events into the chiasmata that join homologous chromosomes together following desynapsis; defects in SC formation are universally followed by the appearance of univalent chromosomes in diakinesis nuclei despite the fact recombination is initiated at appropriate levels [32]. SYP-1, SYP-2, and SYP-3, are all required for synapsis and all localise to the interface between paired homologous chromosomes at pachytene; mutants in any of these components abrogate synapsis while leaving chromosome axes intact, suggesting they are structural components of the central region [15–17]. Furthermore, all three SYP proteins show a mutual co-dependence in their integration into the central region; loss of any one of the components reduces the localisation of the other two to one or two nuclear aggregates. SYP-3, however, shows a discernibly early association with chromosome axes relative to SYP-1 and 2, suggesting it may participate in restricting the localisation of the others to pairs of axes understood to be homologously aligned [17]. The assembly of the central region and chromosome synapsis per se depends on proper chromosome axis morphogenesis. A major constituent of the meiotic chromosome axis is the highly conserved cohesin complex – consisting of an SMC1/SMC3 heterodimeric ring anchored together by the meiosis-specific subunit REC-8 in association with SCC-3 – which mediates sister chromatid cohesion following premeiotic DNA replication [33]. Loss of any of the nematode cohesin components results in sister chromatid cohesion defects that abrogate synapsis [34–36]. Sister chromatid cohesion is required for the recruitment of the meiosis-specific axis component HIM-3 and its paralogues: HTP-1, HTP-2 and HTP-3, which form a family of proteins required for presynaptic alignment and homologous synapsis, in addition to functioning in the regulation of recombination. HIM-3 is thought to represent the orthologue of budding yeast Hop1p, and upon entry into meiotic prophase localizes to developing axes and remains detectably associated with all chromosomes until the metaphase I-anaphase I transition [37]. In the absence of HIM-3 chromosomes (and PCs) fail to achieve presynaptic alignment and synapsis, indicating that the establishment of axis-mediated chromosome architecture is a necessary prerequisite to the alignment process or to homologue recognition itself [27]. Furthermore, recruitment of the SC components SYP-1, SYP-2, and SYP-3 requires HIM-3 at chromosome axes [16, 17, 27], reflecting a structural requirement for HIM-3 in supporting the polymerisation of the central region. HTP-3 also localises to meiotic chromosome axes from the earliest meiotic stages indicating it is a chromosome axis component [18, 38], but unlike HIM-3 is associated with the chromatin of premeiotic nuclei. HTP-3 forms complexes with HIM-3 and is required for HIM-3 recruitment; loss of HTP-3

recapitulates *him-3* loss-of-function phenotypes, indicating a role for HTP-3 in homologue alignment and synapsis that are effected through HIM-3 recruitment [38]. While HTP-1 and HTP-2 share <30% protein sequence identity with HIM-3 and HTP-3, they have 82% protein sequence identity with each other, raising the possibility of functional redundancy and hindering the acquisition of localisation data; nevertheless, genetic analysis has identified functions for the proteins in early meiotic events at the junction of homologue alignment and synapsis [39, 40]. An enduring conceptual problem of homologue pairing addressed by HTP-1 has been how the SC assembly is controlled to ensure that only homologously aligned chromosome pairs are rewarded with synapsis, given that like other organisms, SC can readily form between nonhomologous DNA sequences in *C. elegans* [12, 39, 40] and is highly processive once initiated [18]. In the absence of *htp-1*, homologous chromosomes undergo extensive nonhomologous synapsis [39, 40] and the central region component SYP-1 is precociously loaded to leptotene/zygotene chromosome axes that have not yet aligned with their homologues [39], suggesting that the function of HTP-1 is to inhibit or physically block the association of central region components with unaligned axes. *htp-1* mutants also display defects in presynaptic alignment which may originate in an independent function for the protein in the process itself, or is a secondary consequence of the inappropriate early association of a heterogeneous population of SC components with axes undergoing alignment. While HTP-1 is a negative regulator of synapsis, HTP-2 functions, alone or redundantly with HTP-1, in positively regulating an early step in SC formation [39]. In the absence of both proteins, chromosomes fail to synapse and the precocious association of SYP-1 with axes undergoing alignment is eliminated, despite the fact that SYP-1 and SYP-2 inappropriately associate with unsynapsed chromosomes at pachytene. This suggests that specifically at leptone/zygotene the two proteins have antagonistic functions in synapsis (with HTP-1 blocking SC assembly between unpaired chromosomes and HTP-2 promoting SC initiation) or that HTP-2 functions alone or redundantly with HTP-1 to promote CE assembly once pairing between homologous chromosomes has been attained. Synapsis between homologous chromosomes is also monitored by PCH-2, a conserved ATPase required for the pachytene checkpoint in budding yeast that in the nematode is required for the activation of a synapsis checkpoint triggered by unsynapsed PCs, leading to apoptosis [41].

Meiotic Recombination: From DSB to Crossover

The intricate choreography that leads to homologously synapsed chromosomes is in the majority of meioses a necessary prelude to the essential task of generating chiasmata, the visible manifestation of crossing over between homologues [42]. Like other organisms, most if not all recombination in *C. elegans* initiates with the formation of

programmed double-strand breaks catalysed by the topoisomerase-like transesterase SPO-11 [5]. While Spo11 itself is highly conserved, meiotic DSB formation also requires other factors that show little conservation; in addition to Spo11p, for example, nine other genes are essential in budding yeast for DSB formation, and six of these have no homologues outside of ascomycetes [43]. In *C. elegans*, meiotic recombination initiation requires at least three other proteins in addition to SPO-11; although a direct method for assessing DNA breaks in the nematode is not yet available, indirect assays support a requirement for these proteins at the level of meiotic DSB formation. Loss of the chromatin-associated protein HIM-17 eliminates recombination initiation and correlates with a defect in the timely accumulation of histone H3 methylation at lysine 9 on prophase chromatin [44], suggesting that like in *S. cerevisiae*, chromosomal competence for SPO-11-generated DSB formation requires modification of chromatin structure [45]. A further role for chromosome structure is suggested by the failure to form DSBs in the absence of the HIM-3 paralog HTP-3 [38]. HTP-3 forms complexes with the repair component MRE-11, which like in budding yeast also appears to be required for DSB formation per se, suggesting that HTP-3 and MRE-11 form a pre-DSB complex required for meiotic DSB formation. HTP-3 localization to meiotic chromosome axes is not required for DSB formation, and instead correlates with the association of the protein to the chromatin of premeiotic nuclei, suggesting that it is this population of HTP-3 that is required for DSB formation. Similarly, emerging evidence from yeast suggests that competency to form meiotic DSBs may be linked to changes in chromatin conformation that tightly follow premeiotic DNA synthesis [46], suggesting it precedes full meiotic chromosome axes formation.

Once formed, the ends of Spo11-generated meiotic DSBs are processed in a series of steps using core components that show wide conservation throughout eukaryotic systems [43], consistent with conservation of this pathway [47]. First, the 3′ end of the DSB site is processed to generate an ssDNA tail through the combined activity of the repair complex MRX (consisting of Mre11, Rad50, and Xrs2/Nbs1); a complex that functions in DNA damage repair per se, and that has additional functions during yeast meiosis, including DSB formation, and checkpoint signalling [48]. *C. elegans spo-11*, *mre-11*, and *rad-50* mutants (no direct nematode homologue of Xrs2/Nbs1 has yet been identified) fail to form chiasmata and instead of the six bivalents observed in wild-type diakinesis nuclei, twelve univalents form, consistent with a conserved function in meiotic recombination [5, 49, 50]. Following resection of the 3′ end of the DSB, the resulting ssDNA tails are bound by members of the RecA/Rad51, resulting in a nucleoprotein filament that functions in the invasion and search for homologous sequences to form the heteroduplex DNA structure central to homologous recombination (HR) [51]. Full realization of eukaryotic Rad51 recombinase activity requires the intervention of additional components, including Rad52 in yeast and the tumour suppressor BRCA2 in higher eukaryotes [47]. BRC-2 (ceBRCA2) has been shown to facilitate the loading of RAD-51 onto processed meiotic DSBs [52];

mutants in either component result in severe chromosomal abnormalities and chromatin decondensation defects at diakinesis that are dependent on SPO-11 generated DSBs [52–54], indicating that they are required for all possible HR-mediated repair outcomes. Meiotic DSB repair using the homologue can occur with or without the exchange of flanking sequences to generate crossovers or noncrossovers, respectively, or by using the sister chromatid as a repair template [55]. The crossover outcome during meiotic recombination in *C. elegans* appears to be exclusively dependent upon the activity of HIM-14/MSH-4 and MSH-5, two components related to members of the MutS mismatch repair family [56, 57] that are required for the formation of only a subset of crossovers in budding yeast. While their specific mechanism of action is unknown, loss of either component results in the persistence of RAD-51 foci into inappropriately late stages followed by a failure to form chiasmata [16]. These results collectively suggest that the repair of the recombination intermediate stalls at some point after RAD-51 nucleoprotein filament formation and ultimately resumes using the noncrossover or sister chromatid exchange pathway upon the signal to exit pachytene. Failure to repair meiotic recombination intermediates by this stage activates a widely conserved DNA damage checkpoint that requires the *C. elegans* homologues of the *S. pombe* checkpoint proteins *rad-1*$^+$and *hus1*$^+$, resulting in p53-dependent apoptosis [58, 59].

During meiosis, recombination must take place in the context of the SC, which is required for the maturation of nascent recombination events into crossovers [16]. A functional link between SC assembly and crossover formation is established by ZHP-3, an SC-associated component related to budding yeast Zip3p that, like HIM-14/MSH-4 and MSH-5, is required for crossover formation [60]. ZHP-3 localizes specifically to mature SCs and in its absence synapsis is unaffected and RAD-51-marked recombination intermediates appear, yet chiasmata fail to form, thereby implicating the protein in promoting the crossover outcome of recombination [60]. In addition to supporting proteins functioning in processing of recombination intermediates, the SC, and specifically the chromosome axes, participate in the barrier to the use of the sister chromatid as a repair template during meiotic recombination, thereby promoting crossover formation. For example, RAD-51-marked recombination intermediates appear on time but inappropriately persist until pachytene exit in *syp* mutants in which chromosome axis components like HIM-3 load normally, but chromosomes fail to synapse [16, 17]. Loss of HIM-3 also results in asynapsis, however, the kinetics of disappearance of RAD-51 foci mimics the wild–type pattern and twelve intact univalents appear at diakinesis, suggesting that in the absence of HIM-3 the constraint to recombinational repair with the sister chromatid is alleviated [27].

Studies from a wide range of organisms indicate that the number and distribution of crossovers is regulated during meiosis to ensure the formation of at least one between chromosome pairs, and to place it in regions where it is maximally capable of facilitating chromosome segregation at the first meiotic division [42]. Crossover interference, or the probability that one crossover will occur in the vicinity of another,

is thought to underlie the observation that most organisms show multiple crossovers that are widely spaced [61]. In *C. elegans*, only one crossover forms between homologues [62], indicating that crossover interference is largely complete and that only one recombination intermediate per chromosome pair will mature to a chiasma, even in the case of end-to-end fusions of whole chromosomes that physically double or triple chromosome length [63]. Another facet of control exists in the form of a robust intrachromosomal effect that can drive chiasma formation into small regions of homologous synapsis in the case of rearrangement heterozygosity [10, 64]; in individuals heterozygous for an inversion in the right half and a translocation in the left half for example, the frequency of crossing over in the remaining homologously synapsed region is increased 20-fold to approach levels predicted for the entire length of the chromosome. Both phenomena support the interpretation that *C. elegans* possesses a chromosome-wide interference mechanism that operates in continuous segments of homologous synapsis. In addition to the number of exchange events, the position of these events is also controlled to occur in the terminal third of the chromosome ends [65] and this meiotic distribution of exchanges requires the *rec-1* gene product [66].

Chiasma Formation and Specification of Kinetic Activity

Why is the number and location of crossovers under such tight regulation in *C. elegans*? A likely culprit is the specification of meiotic kinetochore activity. Like other nematodes [67], *C. elegans* mitotic chromosomes are holocentric and microtubules attach along the poleward face of each chromatid to a structure resembling the trilaminar structure of monocentric chromosomes [68]. This arrangement, however, is incompatible with the segregation of recombined sister chromatids, and at meiosis the kinetochore is restricted to one end of sister chromatid pairs, through direct insertion of the microtubules into the chromatin [69]. The specification of kinetic activity to one end is temptingly related to the remodeling of the bivalent upon pachytene exit in response to chiasma formation [70, 71]; loss of the SC components SYP-1/2 from the chromosome arm to one side of the chiasma correlates with the acquisition of kinetic activity by this arm at the first meiotic division. The retention of SYP-1/2 by the other arm of the bivalent correlates with recruitment of the Aurora-like kinase AIR-2 [71], which directs loss of sister chromatid cohesion through the phosphorylation of the cohesin subunit REC-8 and its subsequent cleavage by separase [72]. Sister chromatid cohesion must be disrupted in two steps, first along chromosome arms during meiosis I to release the recombined chromosome arms, and finally at the centomere at meiosis II to segregate sister chromatids [33]. Consequently, the single crossover on each *C. elegans* chromosome appears to provide a point of reference on the symmetric prophase chromosome that ultimately functionally reconfigures the chromosome into an asymmetric configuration in which kinetic activity is assigned and the stepwise loss of chromatid cohesion can proceed.

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Homolog Pairing and Segregation in *Drosophila* Meiosis

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Abstract

Pairing of homologous chromosomes is fundamental to their reliable segregation during meiosis I and thus underlies sexual reproduction. In most eukaryotes homolog pairing is confined to prophase of meiosis I and is accompanied by frequent exchanges, known as crossovers, between homologous chromatids. Crossovers give rise to chiasmata, stable interhomolog connectors that are required for bipolar orientation (orientation to opposite poles) of homologs during meiosis I. *Drosophila* is unique among model eukaryotes in exhibiting regular homolog pairing in mitotic as well as meiotic cells. I review the results of recent molecular studies of pairing in both mitosis and meiosis in *Drosophila*. These studies show that homolog pairing is continuous between pre-meiotic mitosis and meiosis but that pairing frequencies and patterns are altered during the mitotic-meiotic transition. They also show that, with the exception of X-Y pairing in male meiosis, which is mediated specifically by the 240-bp rDNA spacer repeats, chromosome pairing is not restricted to specific sites in either mitosis or meiosis. Instead, virtually all chromosome regions, both heterochromatic and euchromatic, exhibit autonomous pairing capacity. Mutations that reduce the frequencies of both mitotic and meiotic pairing have been recently described, but no mutations that abolish pairing completely have been discovered, and the genetic control of pairing in *Drosophila* remains to be elucidated.

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Alignment and pairing of homologous chromosomes is a nearly universal feature of early prophase of meiosis I and is essential for the segregation of homologs into separate gametes or spores during meiosis I and hence for sexual reproduction. [Note: in accord with recent usage, I will use the term 'pairing' to refer to the close juxtaposition of allelic sequences on different (usually homologous) chromosomes, as detected by labeled nucleic acids or locus-specific fluorescently tagged chromosomal proteins. Associations between homologs in which the pairing status of alleles is not known or is not relevant will be referred to by other specific terms, such as alignment or synapsis, or by the general term 'conjunction', which refers to homologous junctions of undetermined nature.] In most eukaryotes, homolog segregation is mediated by a conserved, recombination-dependent pathway that I shall refer to as the 'chiasmate' pathway. In

this pathway, homologs usually enter meiosis unpaired and must undergo a 'homology search' that commences shortly after the completion of meiotic S phase and that results initially in their rough alignment [1, 2]. Alignment is followed, in most cases, by intimate, widespread pairing and by 'synapsis', the formation of an elaborate zipper-like structure, the synaptonemal complex (SC), that connects aligned homologs from end to end [3, 4]. In early prophase I, paired homologs recombine with each other by a conserved mechanism involving repair of double strand breaks (DSBs) induced by the Spo11 endonuclease [5]. After the completion of recombination in mid-prophase I, intimate pairing is lost and the SC is disassembled, but homologs remain connected at discrete sites known as chiasmata, which are thought to be the cytological manifestations of crossovers [6]. The stability of chiasmata depends upon sister chromatid cohesion near the crossover site [7]. Removal of arm cohesion triggers dissolution of chiasmata and segregation of homologs at anaphase I.

Meiosis is, overall, a highly conserved process, but significant variations have been described and are especially prominent in *Drosophila*. Unlike in most eukaryotes, homologs in *Drosophila* (and other dipterans) are paired to a significant degree in virtually all cell types, including both germline and somatic mitotic cells [reviewed in 2]. This has led to the suggestion that homologs enter meiosis already paired in *Drosophila*, so that a distinct meiotic pairing mechanism may be unnecessary. Moreover, although the chiasmate pathway plays the major role in segregation of the three large bivalents in female meiosis, it shares the stage with two distinct 'achiasmate' segregation pathways, one in female meiosis and the other in male meiosis. The female achiasmate system is responsible for segregation of the small 4th chromosome pair, which never recombines, and serves as a backup system for segregation of occasional non-exchange X, 2nd or 3rd chromosome bivalents [8]. Although the achiasmate segregation mechanism is not fully understood, partner choice has been shown to depend on heterochromatic pairing [8–10]. In male meiosis, crossing over, synapsis and chiasmata are completely absent yet the homologs pair and segregate with exceptional regularity [2]. Stable connections between achiasmate homologs are mediated by two proteins, Stromalin in Meiosis (SNM) and Modifier of mdg4 in Meiosis (MNM) that co-localize to all four bivalents throughout meiosis I until anaphase, when they disappear simultaneously [11]. How they perform this function is not understood.

The role of cis-acting pairing sites has received considerable attention in *Drosophila* meiosis research. The best-characterized example of site-specific pairing is the X-Y bivalent in which pairing is restricted to the rDNA locus [12]. A priori, it seems plausible that achiasmate homologs might rely generally on specific pairing sites since, in the absence of recombination, there is no obvious reason for chromosome-wide pairing; stable pairing at a single, fixed site should suffice to ensure regular segregation. A recent model of this type postulates fixed heterochromatic pairing sites on each autosomal arm that are indifferent to homology and that mediate achiasmate segregation in male meiosis [13]. A predominant role of pairing at specific

sites has also been proposed to account for the fact that translocations act as dominant region-specific crossover suppressors in female meiosis. In this model, pairing and crossing over within a chromosome region are dependent upon physical contiguity of sites that flank the region and that mediate proper alignment of the intervening sequences [14].

In this review, I will summarize recent findings from molecular analyses of pairing in *Drosophila* and will discuss their implications with respect to the mechanism(s) of homolog pairing and segregation in meiosis. I will focus on three questions: (1) whether meiotic pairing is in any way distinct from mitotic pairing, (2) whether pairing and segregation are based on general DNA homology (which I shall refer to as 'egalitarian' pairing) or are driven by interactions between specialized pairing sites, and (3) what we have learned about the genetic requirements for pairing in its different cellular contexts.

Is There such a Thing as 'Meiotic' Pairing in *Drosophila*?

Mitotic Pairing

A unique aspect of pairing in *Drosophila* is that it is not restricted to meiotic cells. Alignment of homologs during mitotic metaphase and anaphase in somatic and germline cells in *Drosophila* and other dipterans was reported by cytologists nearly a century ago [15, 16]. More recently, molecular studies have confirmed and extended these observations. Several studies using FISH probes have shown that homologous loci are paired at frequencies ranging from 40–80% throughout most of the cell cycle in nearly all cell types in vivo and in cell culture [17–23]. The only significant exceptions are early cleavage-stage embryonic nuclei in which the cell cycles are extremely rapid and lack gap phases. Pairing initiates in embryonic nuclei at cycles 12–13 when the cell cycle slows down and begins to include gap phases and increases in frequency as the cell cycle lengthens [17, 21]. An unanswered question is whether pairing is interrupted at a particular stage of the cell cycle. Reductions in pairing have been reported in embryonic nuclei and in larval neuroblasts, but at different stages: anaphase in the embryonic nuclei and S phase in the larval neuroblasts [17, 19].

Male Germ Cells

In *Drosophila* testes, spermatogonia and spermatocytes develop within cysts in which the individual cells are interconnected by ring canals and traverse the cell cycle in synchrony. Each cyst derives from a primary spermatogonium that results from an asymmetric division of a germline stem cell (GSC). Four mitotic divisions precede the meiotic divisions. Consequently, pre-meiotic cysts consist of 1, 2, 4 or 8 spermatogonia and meiotic cysts consist of 16 primary or 32 secondary spermatocytes. Primary spermatocytes enter meiotic S phase almost immediately after the completion of the last spermatogonial division, complete DNA replication in approximately 3 hours and

spend the next four days in a lengthy G2/prophase I in which cell and nuclear volume increase approximately 25-fold [24].

A single-locus fluorescent tagging assay involving an ectopically expressed GFP-LacI protein was used to analyze pairing at 13 sites in autosomal euchromatin marked by transgenic insertions of arrays of *lac*O repeats [13]. In this assay, GFP-LacI is recruited to the *lac*O arrays and results in discrete GFP spots, the numbers of which are indicative of the pairing status of the tagged region. In males homozygous for any of the 13 *lac*O insertions, approximately 50% of spermatogonia exhibited one GFP-LacI spot and the remaining 50% exhibited two spots, indicating that the homologous tagged regions were paired about half of the time. No obvious loss of pairing was observed at any stage of the mitotic cell cycle. Although no cell-cycle stage markers were used in this study, even a fairly brief loss of pairing could have been detected because of the high degree of within-cyst synchrony. Thus, homologs are paired at a substantial frequency at all stages of the cell cycle in the pre-meiotic mitotic divisions in the male germline.

Homolog pairing frequencies increased uniformly to approximately 95% at all tested sites in cysts of early spermatocytes. This transition coincided approximately with the transition from mitosis to meiosis as the increased pairing frequencies were observed in all 16-cell cysts, even the youngest ones in which the cells cannot be distinguished morphologically from spermatogonia. Importantly, no loss of pairing was observed in late gonial or early spermatocyte cysts. Thus, homologs apparently enter meiosis already paired to a significant extent and do not have to 're-pair' at the mitosis-meiosis transition. However, meiotic pairing is not a mere continuation of mitotic pairing as pairing frequencies are uniformly much higher in spermatocytes than in spermatogonia. This indicates that there are meiosis-specific pairing factors that either replace, or supplement and enhance, the factors responsible for mitotic pairing in the male germline.

High pairing frequencies are maintained until the middle of meiotic prophase I, at which time the chromosomes undergo a reorganization that results in the mutual separation of the three major bivalents into separate nuclear domains or 'territories'. This process continues throughout the remainder of prophase I and in mature spermatocytes the major bivalents appear as three chromatin masses confined to widely separated domains in the periphery of the nucleus [24]. Near the time the territories become fully resolved in mid-prophase, homologous pairing and sister chromatid cohesion are suddenly and permanently lost throughout the euchromatic regions of the genome, resulting in the presence of four separate GFP foci that subsequently diffuse independently of one another but remain confined to their common territory [13]. Thus, although mitotic and meiotic pairing appear continuous in the male germline, pairing in spermatocytes involves two dramatic transitions that have no parallel in mitotic cells: a transition from moderate to high pairing frequencies at the onset of meiosis followed by a genome-wide loss of euchromatic pairing in mid-prophase I.

Female Germ Cells

Results of two recent molecular studies of pairing in the female germline, one using FISH [25] and the other the GFP-LacI method [26], are consistent with the suggestion that homologs enter female meiosis already paired, but also suggest that meiosis or germline-specific factors may enhance pairing. In these studies, pairing frequencies in excess of 90% were detected at several euchromatic sites both in nuclei that had initiated synapsis and in pre-synaptic nuclei which included an undetermined mix of mitotic and early meiotic nuclei. In the FISH study, similar pairing frequencies were also measured in nurse cells (the non-meiotic sister nuclei of oocytes) in cysts in which the oocyte was in zygotene/early pachytene.

Asynaptic mutations in *c(3)G*, which encodes a component of the SC central elements [27], were found to sharply reduce pairing frequencies, relative to wild-type, at multiple euchromatic sites in nuclei of all germ cells, including zygotene/pachytene cells, mixed late mitotic/early meiotic cells, and nurse cells [25, 26]. Moreover, the magnitude of the reduction was similar in all stages, despite the fact that C(3)G expression is detected only in zygotene/pachytene-stage cells. This finding indicates that C(3)G must be expressed at low levels in pre-zygotene oocytes and nurse cells and that it functions as a potent pairing enhancer in those cells. This could indicate a synapsis-independent function of C(3)G in pairing, although the alternative that the 'presynaptic' cells contain stretches of SC too short to detect cytologically could not be ruled out. In either case, these results are consistent with the suggestion that 'meiotic' pairing factors act to enhance mitotic pairing frequencies early in germline development.

Heterochromatin versus Euchromatin

In mitotic cells, the measured pairing frequencies are similar at heterochromatic and euchromatic loci in vivo, but slightly lower at heterochromatic than euchromatic loci in cell culture [17, 18, 23]. If meiotic pairing is simply a continuation of mitotic pairing, the distribution of pairing capacity along chromosomes should be similar in mitosis and meiosis. A FISH study of synapsed chromosomes during early meiotic prophase in oocytes found no difference in pairing frequency between euchromatic and heterochromatic X chromosome loci [28], consistent with evidence that heterochromatic regions synapse as completely as euchromatic regions [25, 29]. Interestingly, later in meiotic prophase I, in the extended arrest period during which oocytes are rapidly growing, heterochromatic regions remain paired but euchromatic regions do not [9]. This persistent heterochromatic pairing has been linked to the achiasmate segregation mechanism in females, which relies primarily on heterochromatic rather than euchromatic homology for segregation specificity [8–10].

In male meiosis, direct data on heterochromatic pairing are very limited, because all 13 *lac*O insertions assayed in the GFP-LacI experiment were euchromatic. However, in a separate experiment, Vazquez et al. [13] used a fluorescent centromere marker (CID-GFP [30]) to characterize the pairing dynamics of centromeres. They

found a surprisingly complex pairing pattern very different from that of euchromatic loci. Homologous centromeres pair transiently during mid-prophase I, as indicated by the presence of one CID-GFP spot per territory, shortly after the resolution of chromosome territories and the loss of euchromatic pairing, but are mostly unpaired both in early prophase I (when euchromatic alleles are paired) and throughout late prophase I (when euchromatic alleles are also unpaired) [13]. However, nuclei with more than 8 CID-GFP spots are never seen in primary spermatocytes, indicating that cohesion of sister centromeres, unlike that of euchromatic arms, is retained throughout meiosis I. Thus the pairing (and cohesion) dynamics of one component of heterochromatin, the centromere regions, differs significantly from that of euchromatin.

Heterochromatic pairing in male meiosis has been addressed indirectly in several cytogenetic experiments involving chromosome rearrangements [2]. Most of these experiments used segregation as the readout, but in one, chromosome association patterns in late prophase were also assayed [31]. The unanimous conclusion of these experiments is that homology for autosomal heterochromatin has no impact on chromosome association or segregation patterns. Moreover, although the X and Y pairing sites are located in heterochromatin, non-rDNA heterochromatic homologies between the X and Y have proven ineffective in mediating X-Y pairing and segregation [12]. Thus, unlike in mitosis and female meiosis, pairing capacity effective in directing male homolog segregation is generally absent from the heterochromatin.

Does Pairing in *Drosophila* Rely Primarily on Specific Sites?

Mitosis

Mitotic pairing is clearly not exclusive to certain sites as it has been detected at every locus that has been assayed. These observations do not rule out dependence of mitotic pairing on a few predominant pairing sites but the available data indicate that mitotic pairing capacity is locally autonomous rather than driven by interactions between specific, sparsely distributed sites. The data are of two types. First, a multilocus FISH analysis indicated that loci on chromosome 2 pair independently of one another. A locus could be paired even if flanking loci on the same arm were unpaired, and vice versa [18]. A similar conclusion was reached for multiple euchromatic loci on chromosome arm 3R [21]. Second, rearrangement heterozygosity does not eliminate pairing. Pairing frequencies in heterozygotes for a translocation of a segment of chromosome arm 3R to the X chromosome were reduced by about half (60–70 to 25–40%) within the translocated segment but were unaffected at sites near but outside the translocation [21, 22]. A particularly dramatic example of pairing autonomy of a small chromosome region is provided by a transposition of a small segment of distal 2R, including the *bw* gene, to the proximal 2nd chromosome heterochromatin which failed to even marginally reduce pairing frequencies at the *bw* locus [20].

Female Meiosis

Two studies addressed the degree to which euchromatic sequences pair and synapse autonomously in female meiosis by examining the effects on pairing frequencies of heterozygosity for chromosome rearrangements: reciprocal translocations involving chromosome 3 in a FISH study [25], and the multiply inverted X chromosome 'balancer' FM7 in a GFP-LacI study [26]. In both cases, the rearrangements were chosen because they had previously been shown to act as dominant suppressors of crossing-over; the motivation was to test the hypothesis that the rearrangements suppress crossing over by reducing or eliminating homologous pairing and synapsis in the affected regions [14]. The results of both studies strongly contradicted the pairing hypothesis. In the FISH study, pairing frequencies in two different 3rd-chromosome regions were virtually unaffected by heterozygosity for translocations that severely reduce recombination in those regions, even at a site in close proximity to one of the breakpoints. In the GFP-LacI study, pairing frequencies at several X chromosome sites were only modestly reduced in FM7/X females relative to X/X females. Similar modest reductions were observed in mixed mitotic/early meiotic (presynaptic) cells. Synapsis was also found to be largely unaffected by the rearrangements, although minor abnormalities were observed near the breakpoints. Thus, the effects of heterozygous rearrangements on crossing over are not due to disrupted pairing or synapsis and these effects remain unexplained. More importantly for present purposes, these studies demonstrated that meiotic pairing in *Drosophila* females is not controlled by one or a few master pairing sites on each arm. Instead, autonomous and robust meiotic pairing capacity is widely distributed along chromosome arms.

Male Meiosis – Autosomes

The GFP-LacI study described above showed that meiotic pairing occurs at uniformly high frequencies throughout the autosomal euchromatin, consistent with egalitarian pairing. However, since molecular studies have thus far been limited to wild-type, the degree to which pairing is locally autonomous remains to be determined. Indirect but strong evidence for egalitarian pairing was obtained in a cytogenetic analysis of 2-Y transpositions (Tp(2;Y)s), involving segments of chromosome 2 ranging in length from less than 500 kb to over half a chromosome arm, inserted into the Y [31]. In males heterozygous for a Tp(2;Y) and for normal X and 2nd chromosomes, the Y and normal 2nd chromosomes share homology for a segment of chromosome 2. Stable association between these segments can result in 'quadrivalents' during late prophase I and in preferential segregation of the Y and normal 2 at anaphase I. Elevated quadrivalent frequencies and preferential Y-2 segregation ratios were observed in males heterozygous for any of ten different Tps involving euchromatic segments, even those with segments less than 500 kb in length, but not in males heterozygous for a Tp involving a large segment of 2R heterochromatin. Quadrivalent frequencies ranged from 10 to 100% and were strongly correlated with the length of the transposed segment. Thus, chromosomes sharing homology for even very limited

segments of autosomal euchromatin pair with and segregate from each other at frequencies proportional to the length of shared homology. These data imply that euchromatic loci pair autonomously and that autosomal pairing is predominantly egalitarian in male meiosis.

An unsolved problem in male meiosis is how the intimate and egalitarian pairing of euchromatic sequences during early prophase I is translated into stable attachments between homologs despite the apparently general loss of pairing throughout the euchromatin at mid-prophase I. Time-lapse observations of cultured spermatocytes show that allelic and sister GFP-LacI spots move into close register with one another when the chromosomes condense at prometaphase I, although they do not re-fuse [13].

In orcein-stained squash preparations, wild-type autosomal bivalents at prometaphase I are not only stably conjoined but also show well-aligned arms [32]. Moreover, as discussed above, homologous pairing patterns in Tp(2;Y) heterozygotes are preserved as stable quadrivalents [31]. It has been suggested that after the loss of euchromatic pairing in early prophase, homolog alignment is preserved by stable pairing at fixed sites in the centric heterochromatin of each autosomal arm [13]. However, there is no direct evidence for such sites and, in light of the evidence that only euchromatic homology is effective in directing pairing and segregation patterns, such sites would have to function entirely passively and non-homologously. Insight into this conundrum may emerge from molecular analysis of heterochromatic loci and from functional analyses of the recently discovered SNM and MNM proteins, which are responsible for maintaining stable connections between homologs after the loss of euchromatic pairing in mid-prophase I [11].

Sequence Specificity of X-Y Pairing

The data described in the preceding paragraphs argues strongly against a predominant role of specific sites in either mitotic or meiotic pairing of the autosomes or the X chromosomes in females. However, X-Y pairing in male meiosis provides a clear example of the dominant role of a specific site. A combination of classical and molecular analyses showed that the X and Y chromosomes pair primarily or exclusively within the rDNA loci, which consist of 200–250 tandem copies of the genes for the 18S, 5.8S and 28S ribosomal RNAs and are located in the central region of the X heterochromatin and near the base of the short arm of the Y. Deletions that remove all of the X chromosome rDNA result in failure of X-Y conjunction and high X-Y nondisjunction. X-Y pairing and segregation are significantly restored by X-linked insertions of transgenes carrying single complete rDNA genes [12]. Since the rDNA loci comprise the only extended region of homology between the otherwise divergent X and Y chromosomes, one might postulate that rDNA-mediated X-Y pairing is just a special case of general homology pairing. However, several studies have shown that the rDNA does more than just provide a region of homology between the X and Y. Rearrangements that provide non-rDNA homologies between the X and Y, such as

Y-linked duplications of repetitive or unique sequences derived from the X, are ineffective in mediating pairing and segregation in the absence of rDNA homology [12]. Moreover, pairs of identical mini-X chromosomes which contain more than 1 Mb of proximal X heterochromatin and approximately 300 kb of distal X euchromatin but which lack rDNA segregate randomly from each other in male meiosis, whereas the same mini-X chromosomes with single insertions of a construct carrying only 12 kb of rDNA pair and segregate from one another at significantly elevated frequencies [10, 33]. Thus rDNA repeats have special pairing properties that are lacking in most or all other sex-chromosome sequences.

Fine-structure studies have revealed an additional level of specificity to the pairing capacity of rDNA repeats. Transgenes that carry rDNA fragments that include arrays of 240-bp repeats from the intergenic spacer (IGS) regions of the rDNA can restore pairing ability to rDNA-deficient X chromosomes, whereas transgenes that lack 240-bp repeats but carry other rDNA fragments, even much larger ones, cannot [12]. A recent study involving mini-X chromosomes showed that the basis for this specificity is the ability of 240-bp repeat arrays to recruit the homolog pairing proteins SNM and MNM to sex chromosomes [33]. In light of these findings and of the evidence that SNM and MNM are also recruited to the autosomes and are essential for stable autosomal as well as X-Y conjunction [11], it is possible that the autosomes also contain sequences with special pairing properties. However, no such sequences have been identified as yet, and the evidence discussed above indicates that, if such sequences are present on autosomes, they are distributed much differently than on the X-Y pair.

Genetic Control of Pairing

An attractive mechanism for egalitarian pairing involves DNA base-pairing mediated by homologous recombination proteins of the RecA family. RecA and its eukaryotic homologs Rad51 and Dmc1 have been shown to be capable of efficient pairing of single strand and duplex DNAs in vitro [1, 34]. In meiotic recombination, the DSBs induced by Spo11 are resected to form long single-strand tails, which are then complexed with Rad51 and Dmc1 to form filaments that are capable of rapidly pairing with complementary duplex DNA and, when sufficient homology is present, forming stable interhomolog connections such as Holliday junctions [5, 35]. In some organisms, the initiation of recombination coincides with the early stages of pairing, and mutations in components of the meiotic DSB pathway have been found to disrupt pairing, although it has proven difficult in practice to discriminate between effects of mutations on initiation versus stabilization of pairing [1, 36]. In *Drosophila*, however, synapsis is required for recombination rather than the other way around, and mutations in components of the meiotic DSB pathway such as *mei-W68* (spo11) or *spnA* (rad51) have no effect on either synapsis in females or on X-Y segregation in males [2, 27, 37, 38]. Thus, it seems unlikely that homolog pairing in *Drosophila* is mediated by

components of the meiotic DSB pathway. However, this does not rule out other nucleic acid-based egalitarian pairing mechanisms.

Transcription-based mechanisms for opening up duplex DNA and/or enabling RNA-DNA interactions provide one group of alternatives. It has been suggested that pairing of the 240-bp rDNA repeats in male meiosis might require transcription of their RNA polymerase I promoter copies [12]. The apparent absence of pairing in non-rDNA heterochromatin in male meiosis is consistent with this suggestion. However, it is unlikely that transcription is a general requirement for pairing as mitotic pairing appears to be indifferent to transcriptional status [17, 18, 21]. It has also been suggested that pairing initiation might be favored at Matrix Attachment Regions (MARs), AT-rich sequences that associate with the nuclear scaffold and can unwind relatively easily [2]. This idea is consistent with the recent observation that depletion or disruption of topoisomerase II, a major component of the scaffold, (but not topoisomerase I) in cultured cells via RNAi or chemical inhibitors results in a 25–50% decrease in pairing frequencies at multiple euchromatic sites [23].

A plausible alternative class of mechanisms involves interactions between chromosomal proteins located at multiple sites, e.g. transcriptional regulatory complexes [21, 22]. Although one such transcriptional regulator, the transvection protein Zeste, has been shown to be dispensible for mitotic pairing, mutations in the chromatin insulator component *Su(Hw)* resulted in a 30% reduction in pairing frequency at some euchromatic loci but not at the repetitive histone cluster in imaginal discs [21, 22]. Joint mediation of pairing by many such proteins with partial redundancy could explain why it has been so difficult to identify pairing-defective mutations in *Drosophila*.

Considerable progress has been made in recent years in the identification of proteins required for pairing-related phenomena in meiosis: synapsis in female meiosis, homolog conjunction in male meiosis and sister chromatid cohesion in both sexes. The limited results thus far suggest that homolog pairing is independent of cohesion and of male meiotic homolog conjunction but that the mechanisms of pairing and synapsis in female meiosis may overlap. Pairing is unaffected in both female and male meiosis by mutations in *ord*, a gene required for meiotic cohesion in both sexes and for generation of tripartite SC [28, 39]. Mutations in *snm* and *mnm* cause premature separation of homologs in late prophase I and random homolog assortment at anaphase I. However, they have no effect on euchromatic pairing in early prophase I or in pre-meiotic spermatogonia [11]. Thus their role appears to be in maintaining stable homolog connections rather than initiating pairing or mediating the intensification of pairing at the onset of meiosis. However, as described above, the pairing and synapsis pathways in female meiosis share at least one genetic component. *c(3)G,* which encodes a component of the SC central element, is required both for synapsis and for normal levels of meiotic and pre-meiotic pairing [25–27]. The effects of *c(3)G* mutations on pairing are less drastic than on synapsis, suggesting the presence of additional pairing factors. It remains to be determined whether other SC components

also contribute to meiotic pairing. In light of the apparent continuity of pre-meiotic and meiotic pairing in *Drosophila*, mitotic pairing factors might also contribute to meiotic pairing.

Summary and Conclusions

Molecular studies of pairing in *Drosophila* support the idea of continuity between mitotic and meiotic pairing in both the female and male germlines. There is no indication that homologs unpair and repair during the transition from mitosis to meiosis, although the methods used could not rule out a very rapid transition of this nature. However, the data also indicate major differences between mitotic and meiotic pairing both in intensity, as reflected in mean pairing frequencies, and, at least in males, chromosomal distribution. Moreover, preliminary data in female meiosis suggest that a meiotic synapsis gene, *c(3)G* may have an independent role in pairing in the early germline. Clearly it will be important to find ways to genetically dissect the mitotic and meiotic pairing processes so that their similarities and differences can be understood.

Except in the special case of X-Y pairing, the molecular data provide little support for the popular idea that *Drosophila* relies on specific sites to pair its chromosomes. At the level of resolution provided by FISH and GFP-LacI assays, pairing appears to be largely egalitarian in mitosis, female meiosis and male meiosis. With the possible exception of male meiosis, the pairing machinery is apparently blind even to the euchromatin-heterochromatin distinction. These data do not allow us to conclude that *Drosophila* pairing is fully egalitarian at the molecular level; high resolution studies in the future may identify short, interspersed sequences that, like the 240-bp repeats for the X-Y pair, play a dominant role locally. Nevertheless, at low resolution, pairing and segregation capacity is widespread along chromosomes.

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The Mammalian Synaptonemal Complex: A Scaffold and Beyond

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Abstract

During the first meiotic cell division (meiosis I), homologous chromosomes pair, synapse, recombine, and segregate, using highly coordinated and tightly regulated mechanisms. The synaptonemal complex (SC), a proteinaceous tripartite structure, plays an important role both as a scaffold for the close juxtaposition of homologous chromosomes and in regulating the overall process of homologous recombination. Specifically, it mediates chromosome synapsis during the lengthy prophase of meiosis I. The SC consists of two parallel lateral elements, one central element, and numerous transverse filaments. Recent genetic studies in mice have provided novel insights into the mechanisms by which the SC regulates meiosis and into the etiology of diseases such as aneuploidy. Even though the tripartite ultrastructure and meiotic functions of the SC are similar in different species, the SC components are not well-conserved at the protein sequence level. This review will focus on the identification, characterization, and functions of the synaptonemal complex proteins in mammals.
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Identification of the Synaptonemal Complex

In 1956, Moses and Fawcett used electron microscopy to identify a tripartite chromosomal structure, now known as the synaptonemal complex (SC), in the meiotic prophase I of spermatocytes in a variety of species such as crayfish, pigeon, cat, and human [1, 2]. SC or SC-like structures are present in prophase I cells in most sexually reproducing organisms [3, 4]. Ultrastructural studies revealed that the SC consists of two lateral elements (LEs) and a central element (CE). LEs are parallel to each other and equidistant to the CE. Numerous transverse filaments (TFs) are located between the LEs and the CE in a perpendicular manner [5]. The assembly and disassembly of SCs correlate with the progression of meiotic prophase I. During the leptotene stage, axial elements (AEs) are assembled and each AE is associated with one pair of sister chromatids. Concurrent with chromosome pairing at the zygotene stage, the AEs of homologous chromosomes are progressively connected by TFs in a zipper-like fashion in a process termed synapsis (fig. 1). At the pachytene stage, autosomal homologues

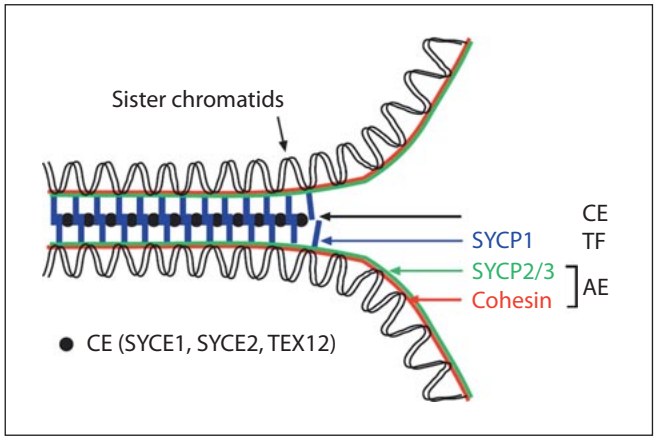

Fig. 1. Schematic diagram of the mammalian synaptonemal complex. A pair of homologous chromosomes at the zygotene stage is illustrated. Each axial element (AE) is formed along the chromosome core of two sister chromatids. Heterodimers of SYCP1 interdigitate as transverse filaments (TFs) in a head-to-head fashion. The SYCE1/SYCE2/TEX12 complex stabilizes TFs within the central element (CE). A series of chromatin loops are attached to the AEs.

are fully synapsed and the XY chromosomes are synapsed only in the pseudoautosomal region [6, 7]. Chromatin is attached to the lateral elements as a series of loops (fig. 1) [8]. In the context of SCs, AEs are referred to as LEs. At the diplotene stage, SCs are disassembled to allow desynapsis and eventual segregation of homologous chromosomes.

In 1985, Heyting and colleagues isolated SCs from rat spermatocytes and subsequently generated a series of monoclonal antibodies against SC proteins for biochemical and cytological analyses [9, 10]. These studies have identified three major mammalian SC proteins: SYCP1, SYCP2, and SYCP3 [10–14]. Immunocytological studies reveal that SYCP1 is a component of TFs, whereas SYCP2 and SYCP3 are components of AEs/LEs. Identification of SC proteins has facilitated the study of not only the SC itself but also other aspects of meiosis such as homologous recombination in mammals since they serve as cytological markers of meiotic progression in mammals.

The Axial Elements (AEs)

During the leptotene stage, an AE is attached to each pair of sister chromatids. It is generally accepted that AEs consist of both synaptonemal complex proteins and cohesin complexes [15, 16]. Two SC proteins, SYCP2 and SYCP3, are known structural components of AEs [12, 14, 17]. In mammals, the mitotic cohesin complex consists of heterodimers of structural maintenance of chromosomes (SMC) proteins, SMC1A and SMC3, and of two non-SMC proteins, RAD21 (previously known as SCC1) and SCC3. In mitosis, cohesin complexes are required for cohesion between sister chromatids, which is established during the S-phase. Most cohesin complexes are released from the arms during the prophase/prometaphase, and cohesion is completely lost in a separase-dependent mechanism at the metaphase-to-anaphase

transition to allow for chromatid segregation [18, 19]. Although cohesin complexes are better known for their role in cohesion of sister chromatids in both mitosis and meiosis, their function in the assembly of AEs during meiosis has increasingly been appreciated [20, 21].

In contrast with mitosis, meiosis consists of one round of DNA replication and two successive rounds of cell divisions. At anaphase I, cohesion is lost from sister chromatid arms but is preserved at centromeres so that only homologous chromosomes are disjoined. At anaphase II, cohesion is lost from the centromeres, which leads to separation of sister chromatids [19]. Thus, mitotic cohesin complexes acquire an extended tour of duty during meiosis. Furthermore, meiosis-specific cohesin proteins (SMC1B, REC8, and STAG3) have evolved. SMC1B is a paralogue of SMC1A [22]. REC8 is related to RAD21 [20]. STAG3 is homologous to SCC3 [23, 24]. While REC8 is conserved from yeast to mammals, SMC1B and STAG3 appear to be restricted to higher eukaryotes such as mammals.

Cohesin proteins constitute part of AEs formed between sister chromatids (fig. 1). REC8 appears on chromatin shortly before the pre-meiotic S phase and forms AE-like structures between sister chromatids [25]. Subsequently, SMC1B, SMC3, SYCP2, and SYCP3 are assembled along the REC8-containing linear structures to form the *bona fide* AEs [25]. At metaphase I, these proteins (SMC1B, SMC3, and SYCP3) are mostly lost from the chromosome arms, but persist at the arms of the metaphase I bivalents and accumulate near the centromeres [25–28]. In contrast, REC8 remains abundant along the chromosome arms until anaphase I and persists near the centromeres until anaphase II. Mitotic SMC1A also localizes to AEs but does not accumulate near the centromeres at metaphase I [22, 29]. STAG3 also appears to be specific to chromosome arms during metaphase I [24].

The long-standing question is whether cohesin complexes are required for assembly of SC proteins (SYCP2 and SYCP3) into AEs. In yeast, REC8 is required for formation of AEs [20]. However, in *Rec8* mutant mice, AEs containing SYCP3 are still assembled, but synapsis occurs between sister chromatids rather than homologous chromosomes [30, 31]. In *Smc1b*-deficient mice, SYCP3-containing AEs are formed but shortened [32]. Therefore, neither REC8 nor SMC1B alone is required for incorporation of SYCP3 (and presumably also SYCP2) into AEs, even though disruption of either cohesin subunit impairs synapsis. Interestingly, SMC1A and SMC3 form complexes with SYCP2 and SYCP3 in spermatocytes [29]. While localization of SYCP2 and SYCP3 on AEs is continuous, SMC1A and SMC3 localize as dots along AEs in pachytene and diplotene spermatocytes, suggesting that SYCP2/SYCP3 are engaged in limited interactions with SMC1A/SMC3. Since SMC1A and SMC3 are also expressed in mitosis, they are likely to be essential for viability. If this is true, it would be difficult or impossible to study their function in meiosis using genetic approaches because the necessary knockout mice would not survive. Therefore, it is still not clear whether SYCP2/SYCP3 are incorporated into AEs through binding to cohesin complexes or direct interaction with chromatin.

Genetic studies demonstrated that SYCP2 and SYCP3 are required for the SC assembly [33, 34]. SYCP3, a small coiled coil protein, forms homotypic dimers or oligomers [35]. When ectopically expressed in cultured somatic cells, SYCP3 can self-assemble into multi-stranded fibers [36, 37]. SYCP3 also interacts with SYCP2 [33, 38]. SYCP2 and SYCP3 colocalize to AEs/LEs. Additionally, SYCP2, but not SYCP3, localizes to axial associations or bridges between LEs within SCs. The functional significance of this observation remains to be elucidated [17]. The evolutionarily conserved C-terminal coiled coil domain in SYCP2 is required for its interaction with SYCP3 [33]. These studies are consistent with a model in which SYCP2 and SYCP3 assemble into AEs as SYCP2-SYCP3 heterodimers and SYCP3 homodimers. *Sycp2* or *Sycp3* mutant mice exhibit meiotic arrest at the zygotene stage, failure in the formation of normal AEs, and impaired chromosomal synapsis [33, 34]. In *Sycp3*-deficient meiocytes, SYCP2 is not localized to axial chromosomal structures (i.e. AEs) [21]. In a sense, the *Sycp3*-null mutant can be treated as a *Sycp2 Sycp3* double mutant. The *Sycp2* mouse mutant is unique in that it lacks only the small coiled coil domain of SYCP2 that is required for binding to SYCP3 [33]. As expected, the truncated SYCP2 protein (termed SYCP2t) is still expressed and is not associated with SYCP3. Importantly, SYCP2t but not SYCP3 localizes to the remnant AEs, whereas SYCP3 forms large protein aggregates in the nucleus, suggesting that SYCP2 is required for incorporation of SYCP3 into AEs. Thus, the *Sycp2* mouse mutant lacking the coiled coil domain is a separation-of-function mutant. SYCP2 has at least two separable functions: association with AEs and binding to SYCP3. Study of the *Sycp2* mutant also revealed a possible hitherto unknown regulatory function for SYCP3 in the association of SYCP2 with AEs [33]. We are currently in the process of generating a *Sycp2t Sycp3* double mutant to study the assembly of SYCP2 and SYCP3 into AEs.

A model for AE assembly has been proposed in which cohesin complexes are assembled as rudimentary AE-like structures between sister chromatids (fig. 1) [4, 19, 21, 25]. Subsequently or simultaneously with some cohesin subunits such as SMC1B and SMC3, SYCP2 and SYCP3 are incorporated into the AE-like structures to form the normal AEs [25]. As shown in the *Sycp3*-null mice, localization of cohesin subunits to axial chromosomal structures is not affected and thus formation of the rudimentary AE-like structures does not require SYCP3 or SYCP2 [21]. Even though the cohesin core enables limited synapsis between homologous chromosomes, synapsis is severely impaired in males lacking SYCP3 [21, 39].

Studies of mouse mutants also revealed additional but distinct functions for SMC1B and SYCP3 in chromatin dynamics [16]. In *Smc1b*-deficient mice, SCs are shortened by half but chromatin loops are twice as long compared to wild type [32]. In *Sycp3*-deficient mice, cohesin cores in spermatocytes or SYCP1 fibers in oocytes are extended twice, whereas the length of chromatin loops does not change [39, 40]. Thus, these studies revealed important roles for cohesin and SC proteins in chromosome compaction.

The Transverse Filaments (TFs)

SYCP1 is the major constituent of TFs. SYCP1 contains a long central coiled coil region of about 700 amino acids that is flanked by globular N- and C-terminal domains [11]. Within the SC, parallel SYCP1 molecules are probably organized as extended homodimers with the same polarity [41, 42]. The C-terminal end of SYCP1 is embedded within LEs, whereas the N-terminal end is located in the central element [41, 42]. SYCP1 molecules are organized head-to-head through the interaction between the N-terminal domains. In a heterologous system, SYCP1 self-assembles into polycomplexes that resemble SCs and importantly, shortening or lengthening of the central coiled coil domain affects the width of polycomplexes accordingly [43].

Although the ultrastructure of SC appears to be similar among eukaryotes, the protein components of TFs, like components of AEs, are not well-conserved at the sequence level. Therefore TF components have been identified independently in different organisms. Known TF components include yeast Zip1, fly C(3)G, and nematode SYP-1 and SYP-2 [44–47]. *Arabidopsis* ZYP1 was identified using a bioinformatics approach based on the structural features of known TF proteins: a long coiled coil domain flanked by globular domains at both ends [48]. However, the molecular mechanism by which transverse filaments connect the two parallel lateral elements remains unknown. SYCP1 has not been shown to interact with any component of LEs such as SC and cohesin proteins [35, 38]. Because the C-terminal domain of SYCP1 is basic and contains S/TPXX motifs, the C-termini of SYCP1 are embedded within LEs, possibly through binding to chromatin DNA [11, 15].

Disruption of the *Sycp1* gene causes sterility in both sexes [49]. In *Sycp1* mutant mice, normal AEs are assembled; homologous chromosomes are aligned but fail to undergo synapsis; DSB breaks are formed but are not efficiently repaired, so crossovers do not take place. Thus, SYCP1 is required for chromosomal synapsis and completion of homologous recombination.

Several studies provide evidence suggesting that phosphorylation regulates disassembly of SCs in some way. Both SYCP1 and SYCP3 are phosphorylated [14, 50]. SYCP3 is recognized as two distinct bands of 30- and 33-kDa on western blots [14]. Two-dimensional electrophoresis resolved SYCP3 into at least 24 spots, suggesting the presence of multiple phosphorylation variants [51]. Furthermore, treatment of rodent pachytene spermatocytes with the phosphatase inhibitor okadaic acid caused rapid dissolution of SCs. Phosphorylation of SYCP1 coincides with its dissociation from chromosomes [50]. However, the kinase responsible for phosphorylation of SYCP1 and SYCP3 is currently unknown.

The Central Element (CE)

Composition of the central element has remained a mystery for nearly five decades. It is generally believed that the central element is formed by interdigitating SYCP1

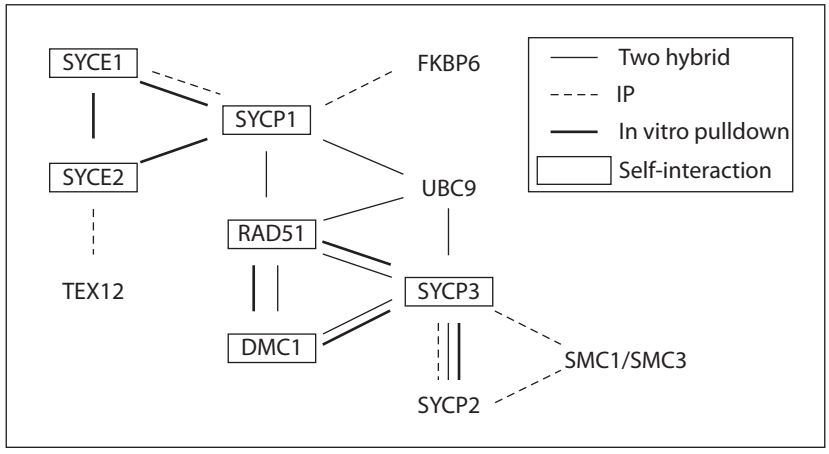

Fig. 2. A molecular interaction network on the mammalian meiotic chromosomes. Known protein-protein interactions are schematically shown as lines. The method by which a particular interaction was demonstrated is depicted with a different line (yeast two-hybrid, immunoprecipitation (IP), and in vitro pulldown). Proteins that interact with themselves are boxed. It is worth noting that the biological relevance of some interactions is not known.

homodimers arranged in a head-to-head fashion from opposing lateral elements. Solari and Moses noted protease-sensitive materials in the CE region in addition to the TFs [52]. Furthermore, tomographic reconstruction of the CE region revealed pillar-like structures connecting the TFs [5]. Only recently, CE components have been identified using a combination of genomic and cytological approaches. In 2005, Costa and colleagues for the first time identified two CE-specific proteins (SYCE1 and SYCE2) in mouse [53]. In 2006, Hamer and colleagues reported a third CE-specific component called TEX12, which was previously identified as a germ cell-specific protein [54, 55]. SYCE1, SYCE2, and TEX12 localize exclusively to the central element. Interestingly, all three CE proteins, like SYCP1, possess coiled coil domains, which are common protein interaction motifs. Therefore, these CE proteins constitute an extended molecular network within the CE (fig. 2). SYCE1 and SYCE2 interact with themselves, with each other, and with the N-terminal region of SYCP1 [53]. TEX12 for its part is associated with SYCE2 but not with SYCE1 or SYCP1 [54]. Localization of all three proteins to the CE is dependent on SYCP1, and it has been proposed that the CE complex (SYCE1, SYCE2, and TEX12) plays a role in the assembly and stability of the SC [54].

Mice deficient in the *Syce2* gene have been generated [56]. The phenotype of the *Syce2* mutant resembles that of the *Sycp1* mutant, providing further support for the essential role of CE-components in synapsis and meiotic recombination. Furthermore, the study of the *Syce2* mutant provided mechanistic insights into the polymerization of SC. In *Syce2* mutant meiocytes, SYCP1 and SYCE1 localize only to small regions, which is consistent with the model where association of SYCP1 dimers at the CE is initially stabilized by SYCE1, but further polymerization of SC requires SYCE2 [56].

SC-Associated Proteins

Besides the major SC components described above, several additional proteins such as HSP70–2, FKBP6, and UBC9 are known to localize to the SC and here are collectively referred to as SC-associated proteins. Studies of these proteins in meiosis revealed the tremendous complexity of the regulation of SC dynamics and suggested that more SC-associated proteins might be identified in the future.

HSP70–2, a testis-specific chaperone, localizes to SCs in pachytene and diplotene spermatocytes but not in fetal oocytes [57]. Targeted inactivation studies showed that HSP70–2 is required for desynapsis of SCs in male mice [58]. Biochemically, HSP70–2 interacts with cyclin B-dependent CDC2 kinase, whose activity triggers the G2/M transition in both mitosis and meiosis. Thus, the essential role of the HSP70–2 chaperone activity in the assembly of CDC2/cyclin B1 might explain the meiotic block in HSP70–2-deficient males [59].

FKBP6 belongs to a protein family that contains a binding domain for an immunosuppressive drug called FK506. FKBP6 colocalizes to SCs with SYCP1 and these two proteins are associated with each other in testis (fig. 2). Disruption of *Fkbp6* causes defective synapsis, abnormal pairing, and misalignment between homologous chromosomes in male but not female mice, resulting in male sterility [60]. FKBP6 is also required for male fertility in rats, since a deletion of *Fkbp6* exon 8 is the causative mutation in the spontaneous *as/as* rats [60].

Recent studies showed that sumoylation plays an important role in the assembly of the SC in yeast [61, 62]. Zip3, a component of the yeast SC, is a SUMO (small ubiquitin-like protein modifier) E3 ligase [61]. Zip1, a yeast TF component, is associated with Zip3-dependent sumoylated proteins to form SCs [61]. UBC9 is a SUMO-conjugating (E2) enzyme that is involved in many cellular processes. UBC9 localizes to SCs in both yeast and mammals [62, 63]. A non-null allele of the yeast *Ubc9* gene impairs chromosomal synapsis [62]. In basidiomycetes, UBC9 interacts with DMC1 and sumoylates DMC1 [64]. In mammals, UBC9 interacts with RAD51, SYCP1, and SYCP3 (fig. 2) [35, 63]. Interestingly, IPO13 (importin 13) mediates nuclear import of UBC9 in mouse meiotic germ cells [65]. These recent studies suggested that sumoylation plays an evolutionarily conserved role in the regulation of meiosis, particularly in the assembly of SCs, from yeast to mammals. However, the possible function of UBC9 in mammalian meiosis remains to be explored using genetic and biochemical approaches.

Functions of the Synaptonemal Complex Proteins

In mammals, the tripartite structure of SCs is essential for chromosomal synapsis and meiotic recombination. During meiosis I, homologous recombination allows reassortment of genes between the parental genomes to generate genetic diversity. On the

other hand, homologous recombination ensures faithful segregation of homologous chromosomes, which are only connected at the sites of crossover referred to as chiasmata after desynapsis. Formation of SCs promotes chromosome compaction and provides close juxtaposition of homologous chromosomes during meiosis I. Chromosome synapsis and homologous recombination are mutually dependent in most species including budding yeast and mammals. Defective synaptonemal complex assembly in *Sycp1*, *Sycp3,* or *Syce2* mutants impairs both chromosomal synapsis and homologous recombination [40, 49, 56, 66]. In all three mouse mutants (*Sycp1*, *Sycp3*, *Syce2*), γH2AX and recombination-related proteins are retained on meiotic chromosomes, suggesting the importance of SC proteins in the efficient execution of recombination-related events.

Several distinct protein complexes/structures in meiosis have been identified: cohesin complex, AE/LE, TF, CE, and the recombination machinery (RAD51, DMC1, etc.). A great deal is known about the composition of each complex and crosstalk between these complexes. For example, the CE complex seems to be attached to the TFs through direct interaction between SYCP1 and SYCE1 (fig. 2) [53, 54, 56]. Interactions between recombination-related proteins (RAD51 and DMC1) and SC components (SYCP1 and SYCP3) have been detected (fig. 2), suggesting a possible physical link between recombination and synapsis in mammals [38]. However, some key questions remain unanswered. First, the cohesin complex is considered as part of AEs/LEs, but it is not clear how SC proteins, specifically SYCP2 and SYCP3, are assembled on top of cohesin cores. Second, TFs are believed to connect with LEs, but how the TF component (SYCP1) interacts with LEs is still unknown.

The evolution of SCs includes elements of abundant diversity as well as great conservation. The tripartite structure of SCs is strikingly conserved among diverse organisms, but their components are highly diverged [3, 4]. For instance, although database searches showed that orthologues of SYCP1, 2, and 3 are present in all vertebrate groups, these three proteins have no apparent corresponding homologues at the amino acid sequence level in budding yeast, *Drosophila*, or *C. elegans*. However, functional homologues are present in these lower eukaryotes, at least for SYCP1 [44–46].

SC proteins might be profoundly involved in human abnormalities such as aneuploidy, infertility, and even tumorigenesis. Aneuploidy in germ cells is a leading cause of pregnancy loss and increases with maternal age [67]. Interestingly, in *Sycp3*-deficient female mice, a subset of oocytes with univalent chromosomes evade meiotic checkpoints, resulting in female fertility with reduced fecundity but increased aneuploidy in female germ cells [66, 68]. Consequently, loss of SYCP3 function causes increased embryo death in female mice [40]. Mutations in SC proteins may lead to infertility in humans. For example, two infertile men have been found to be heterozygous for a nonsense mutation in *SYCP3*, suggesting a possible dominant negative effect [69]. In addition, testis-specific genes tend to be ectopically expressed in human tumors. SYCP1 is expressed in somatic tumors, such as T-cell lymphomas, and thus belongs to a class of so-called cancer-testis antigens. An epitope from SYCP1

can be presented by dendritic cells to $CD4^+$ T cells, making SYCP1 a strong candidate for lymphoma vaccine development [70]. Intriguingly, RNA interference knockdown studies demonstrated that HSP70–2, an SC-associated chaperone protein, is required for tumor cell growth and survival through distinct mechanisms [71, 72].

Opportunities

Undoubtedly, studies of SCs in lower eukaryotes such as yeast, *Drosophila*, and *C. elegans* will continue to provide valuable novel insights, in part due to the power of genetics in these organisms [4]. Study of the mammalian SC will have its own advantages, because of superb cytology, well-defined meiotic stages, relative abundance of material, increasingly amenable genetics, and close relevance to humans. In fact, significant advances in meiosis have already been attributed to mammalian studies. For instance, the TF component (SYCP1) was first discovered in mammals [11]. As another example, CE-specific components were recently found first in mouse [53, 54]. The growing availability of sequenced genomes from a large variety of species will only accelerate the pace of understanding how SCs affect health and reproduction, including meiosis. Rapid advances will likely be made in illuminating the structure of SCs, its assembly and disassembly, and its role in synapsis and homologous recombination over the coming years. A few specific experimental opportunities seem within reach. For example, the stoichiometry of SC components, which would provide a basis for deciphering the molecular organization of SCs, could be determined by biochemical purification of SCs followed by mass spectrometry. No crystal structure of any SC protein has been reported. SYCP3 is a relatively small protein (30 kDa) and thus is ideal for crystallography. Solving the crystal structure of SYCP3 and other SC proteins will greatly advance our understanding of SC assembly and disassembly.

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The Dance Floor of Meiosis: Evolutionary Conservation of Nuclear Envelope Attachment and Dynamics of Meiotic Telomeres

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Abstract

Segregation of the homologous chromosomes is the most important feature of meiosis as it ensures the faithful haploidization of the genome. It essentially depends on an accurate prearrangement of chromosomes that culminates in a precise and unambiguous pairing of the homologs, which in turn is a prerequisite for their correct segregation. Pairing with the right partner is accompanied by, moreover it implicitly requires characteristic chromosomal movements that, remarkably, appear to be driven by the chromosomal ends. In prophase I, telomeres firmly attach to the nuclear envelope and move to congregate in a small cluster, thus trailing homologs into close vicinity, a condition that was suggested to promote homolog recognition and alignment. The evolutionarily highly conserved phenomenon of the telomere driven meiotic chromosome rearrangement is yet known for a long time, but the molecular mechanisms responsible for telomere attachment and their directed movements have remained largely unknown. However, in the recent years significant progress has been made in this issue, which has provided some novel clues about the molecular requirements and function of the characteristic meiotic telomere dynamics.
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Meiosis is a unique type of cellular division by which a diploid cell produces genetically distinct haploid gametes. To reduce ploidy cells are confronted with an exceedingly difficult challenge. Somatic cells that enter a mitotic division have a clear-cut one-to-one prearrangement of the genetic material that is to be separated. During S-phase two identical copies are generated which, by sister chromatid cohesion, are in an unambiguous tightly paired condition as early as replication process takes place. The structurally paired condition allows for attachment of a bipolar spindle that on resolution of cohesion during metaphase-anaphase transition separates the two copies to produce genetically identical daughter cells [1]. This kind of segregation is also valid for the second meiotic division by which in a similar manner sister chromatids are segregated. In

contrast, during meiosis I cells are entrusted with the separation of maternal and paternal chromosomes, which, on entry into meiosis, usually are randomly distributed and structurally distinct from each other. However, to ensure their proper segregation homologs have to identify each other and to assort to precise one-to-one units that are captured by a bipolar spindle attaching monopolar at each single chromosome, thus allowing homologous chromosomes moving apart from each other to the opposite poles in anaphase I [2]. Hence, finding and pairing with the right partner represents the key problem for genome haploidization and therefore embodies the most fundamental processes of meiosis. It is a quite demanding task that is accomplished by a complex series of chromosomal interactions during first meiotic prophase that include alignment, pairing, synapsis as well as recombination of the homologous chromosomes.

Homolog search and pairing are accompanied by vigorous movements of chromosomes that follow a unique but evolutionarily highly conserved choreography. A central characteristic is the formation of the so-called chromosomal bouquet at leptotene/zygotene transition. It represents a kind of directed chromosomal movement that was found to be implicitly necessary for meiotic progression. On entry into meiosis chromosomal ends firmly attach to the nuclear envelope where they start to move to congregate at one pole of the nucleus. Thereby chromosomes are trailed into a bouquet like configuration bringing homologs into close vicinity, a process that is supposed to further interhomolog interactions required for proper pairing and recombination [3]. Despite the wide conservation of bouquet formation and its obvious role in juxtapositioning of the homologs, the molecular mechanisms responsible for telomere attachment and movements in first meiotic prophase are poorly understood. However, great progress has been achieved in the past few years which gave us new insights into these very important processes.

Telomere Behavior during Meiotic Prophase I

Chromosomal rearrangement during meiotic prophase I follows a characteristic and evolutionarily highly conserved sequence. It resembles a tightly conducted 'chromosomal dance' that culminates in clear-cut alignment and synapsis of the homologs. At the beginning of meiotic prophase nuclei and chromosomes initially appear motionless. However, by the end of leptotene nuclei start to rotate and vigorous oscillatory movements of the chromosomes can be observed which last until mid-zygotene and are strongly correlated with the pairing process in the nuclear interior [4]. Thereby chromosomes are brought into a polarized and characteristic bundled configuration known as 'chromosomal bouquet' which represents a very central aspect during leptotene-zygotene transition. This kind of polarized chromosomal gathering is a widespread phenomenon that, with occasionally some slight variations, applies for almost every sexually reproducing eukaryote pointing to a very central role for meiotic progression [for review see 3, 5, 6].

Formation of the chromosomal bouquet appears to be implicitly dependent on and, even more, directed by the ends of the chromosomes. Telomeres, which are usually spread in the nucleus in somatic as well as premeiotic cells, dramatically change their spatial arrangement on entry into meiosis. They migrate to the nuclear periphery, where they get physically attached to the nuclear envelope (NE). Once attached, telomeres start to move along the inner surface of the NE to transiently congregate at one pole of the nucleus. In animals and fungi telomere clustering occurs adjacent to the microtubule organizing center (MTOC), i.e. centrosome or spindle pole body (SPB), respectively [7, 8; for review see 3]. In plants, however, which lack a clearly localized MTOC, telomeres tend to polarize opposite the major concentration of cytoplasmic microtubules [9]. Despite slight differences with respect to the congregation center and, in some cases, to timing (e.g. in *Arabidopsis* an unusual nucleolus-associated telomere clustering is already seen at the beginning of meiotic interphase, a bouquet-like configuration that is maintained until early prophase I [10]), telomere clustering nevertheless represents a conserved common scenario by which the associated chromosomes are trailed into the typical polarized bouquet conformation. At pachytene stage, the bouquet configuration disappears and telomeres, now paired but still remaining attached, redisperse around the inner surface of the NE. Telomere-NE connection lasts until late prophase before telomeres detach from the NE and cells proceed to diakinesis.

The close temporal correlation between telomere clustering and homolog alignment and synapsis led to the suggestion that the unique telomere movements occurring during meiotic prophase are required for facilitating chromosome recognition and stable pairing of the homologs [3, 11–13]. In the past few years, this longstanding hypothesis has found clear support by studies in yeast and, more recently, in higher eukaryotes as well. In mutants that are characterized by the absence of telomere attachment and/or clustering, homolog pairing and recombination are significantly delayed or disrupted [14–21]. Remarkably, bouquet configuration is also achieved in haploids of rye and, subsequently, a considerable amount of synapsis is observed, however mostly of non-homologous nature. Although under this condition each chromosome lacks its normal homologous partner, some homologous-like recombination occurs, whereby chiasmata are preferentially located near telomeric regions [22]. Certainly, formation of a bouquet is not sufficient to ensure pairing and synapsis as evidenced by several mutants that are defective for early recombination events such as formation of double-strand breaks [for review see 23]. Nevertheless, the bouquet mutants demonstrated that telomere clustering is essential for proper meiotic progression, most likely by supporting recognition and alignment of the homologs.

Ultrastructure of Telomere Attachment Sites

Meiotic attachment of telomeres is much more than a loose association with the NE, rather it is a tight structural connection that is formed early in meiotic prophase and

preserved for nearly the whole meiotic prophase I. This became already evident in the very first ultrastructural studies carried out on spermatocytes more than half a century ago. In 1956 Moses could find that axial/lateral elements (AEs/LEs) of the synaptonemal complexes are in continuity with the NE whereby chromosomal ends make intimate contact to the inner nuclear membrane (INM) [24, 25]. Meiotic telomere-NE association appears mechanically exceedingly stable so that it is actually able to withstand harsh spreading techniques [26, 27]. Intensive ultrastructural evaluation of the telomere-NE intersection revealed a characteristic organization of chromosomal ends but also of the nuclear envelope at the sites of attachment. Towards their contact points with the INM AEs/LEs become characteristically thickened. At these sites, the nuclear membranes are more dense than in other regions and both inner and outer nuclear membranes, are crossed by thin fibrils to constitute a direct connection between chromosomal ends and cytoplasmic structures [28–30; see also fig. 1A]. Since these fibrils arise from the nuclear side of chromosomal attachment, then traverse the perinuclear space and emanate into the cytoplasm, it was suggested that material of the lateral elements themselves goes through the membranes to form a sort of 'root' of attachment [29]. This hypothesis, however, was recently qualified by the observation that the formation of AEs/LEs in effect is not required for telomere attachment and their linkage to cytoplasmic structures. In *Sycp3*$^{-/-}$ spermatocytes AEs/LEs are absent, but telomeres nonetheless still attach firmly to the NE. Chromosomal ends of *Sycp3*$^{-/-}$ spermatocytes lack the characteristic conical thickening which uncovered that telomeres attach to the inner nuclear membrane through a flat, disk-shaped attachment plate. These plates in turn are connected to the cytoplasm via the typical membrane traversing filaments that are also seen in the wild type, thus demonstrating that a discrete functional attachment and linker complex is formed even though AEs/LEs are absent [31; see fig. 1B].

Taken together, ultrastructural analysis of the telomere attachment sites revealed a tight linkage between chromosomal ends and the nuclear envelope. Moreover, it disclosed a direct connection between meiotic telomeres and structural elements residing outside the nucleus. Thus, meiotic telomeres and consequently chromosomes appear like 'marionettes in a meiotic puppet theater' attached to nucleocytoplasmic threads and conducted by forces most likely operating from the cytoplasm.

Molecules Involved in Telomere Attachment and Movement

Meiotic attachment of telomeres and their choreographed motions that culminate in the characteristic bouquet configuration are now known for more than one century [32, 33; for review see 3, 5]. Although known for such a long time, the molecular mechanisms that are required for attachment and movement of chromosomal ends remained obscure. Significant progress in this issue came from recent studies that were aimed for identifying the molecules involved in forming the attachment complex

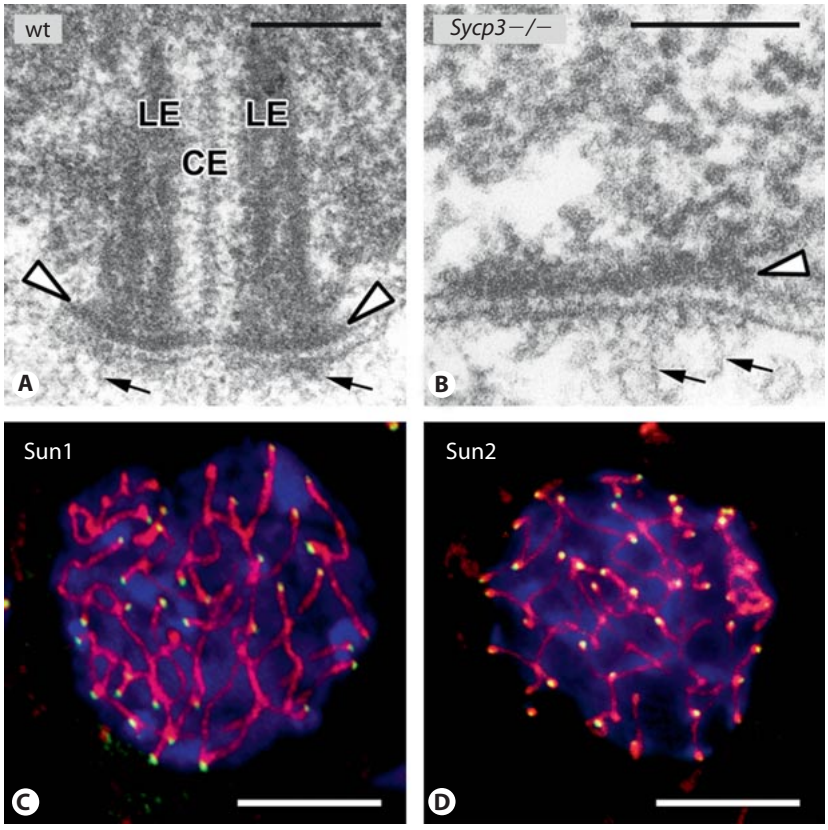

Fig. 1. NE attachment of meiotic telomeres. **A** Transmission electron microscopy of a telomere attachment site in a wild-type mouse pachytene spermatocyte. The two lateral elements (LE) of the synaptonemal complex terminate with a conical thickening at the NE. Electron-dense attachment plates connect the wide end of each LE with the inner nuclear membrane (arrowheads). Thin fibrils arise from the attachment plates, cross both nuclear membranes and emanate into the cytoplasm (arrows). CE, central element. **B** Ultrastructure of telomere attachment in a *Sycp3*$^{-/-}$ spermatocyte. Note that a discrete attachment plate is formed even in the absence of AEs/LEs. **C** and **D** Immunolocalization of SUN-domain proteins (green) in mammalian pachytene spermatocytes. Both, Sun1 (**C**) as well as Sun2 (**D**) show a punctate pattern, whereby their localization is restricted to the NE attachment sites of meiotic telomeres. SCs are stained in red, DNA in blue; scale bars = 10 μm [see also 20, 51]. **A**, **B** are reproduced from [31]; scale bars = 0.2 μm.

and/or more general in regulation of attachment and movement, respectively. Since meiotic chromosomes attach to the NE via their ends, a direct involvement of telomeres themselves is more than overt. Consequently, telomere chromatin was found to be intimately associated with the attachment plates which could be unraveled in mice lacking AEs/LEs [31]. Substantial loss of telomere repeats that is seen in late generations of telomerase deficient mice [34] severely interferes with alignment and recombination which demonstrates that telomere repeats are indeed essential for NE attachment and meiotic progression [35–37]. Further evidence came from studies

analyzing the behavior of synthetic ring chromosomes with or without telomere repeats. Mini chromosomes lacking telomere repeats fail to associate with the NE and do not participate in bouquet formation, while ring chromosomes with telomere repeats behave like normal chromosomes with respect to telomere attachment and clustering [38, 39]. Besides the need of telomere repeats, attachment of chromosomal ends depends on the structural integrity of telomeres as well. In fission yeast telomere associated proteins Taz1, the functional ortholog of the mammalian telomere repeat-binding factors TRF1 and TRF2, and its binding partner Rap1 were found to be essential for telomere attachment and bouquet formation [14, 15, 40]. Furthermore, telomere localized cohesin subunits appear to have, beside their general role in sister chromatid cohesion, a distinct function in attachment and movement. Although having no direct effect on telomere attachment and clustering, Rec8 deficiency in budding yeast causes a significant defect in cluster-SPB association [41]. On the other hand mammalian meiosis specific SMC1β appears to be critical for faithful NE-attachment of telomeres as shown in SMC1$\beta^{-/-}$ mice [42]. However, whether cohesins are required for maintaining the general integrity of meiotic telomeres or might be more directly involved in tethering telomeres to the NE has remained elusive.

Telomere attachment requires specific docking sites within the NE. In fission yeast this job is assigned to a protein named Sad1, which initially was identified as a component of the spindle pole body (SPB) [43]. Sad1 belongs to a novel group of INM proteins that share a conserved C-terminal SUN (Sad1/UNC-84 homology) domain and are found across all eukaryotes. SUN-domain proteins are expressed both in somatic as well as germ cells [44]. SUN-domain proteins interact with various KASH-domain (Klarsicht, ANC-1 and SYNE1 homology) partners, which are located in the outer nuclear membrane (ONM), to form a protein complex spanning both nuclear membranes, hence providing a structural linkage between nuclear components and the cytoskeleton. In somatic cells this nucleocytoplasmic linker complex was found to be crucial for nuclear anchoring, migration, and positioning [for recent overview see 44, 45]. In fission yeast meiosis, Sad1 specifically interacts with KASH-domain protein Kms1 to form such a bridging complex that, on its part, was shown to be crucial for faithful telomere attachment and congregation to the SPB [13, 46, 47]. Recent studies have further demonstrated, that the involvement of SUN-domain proteins in tethering meiotic telomeres to the NE applies not only to fission yeast, but is also apparent in other species including lower as well as higher eukaryotes, pointing to an evolutionarily conserved mechanism of telomere attachment among eukaryotes. In budding yeast, for example, anchorage of telomeres to the NE is mediated by SUN-domain protein Mps3. Mps3 in turn binds ONM protein Mps2, thus forming a nucleocytoplasmic bridge that links telomeres directly to the SPB [18, 48, 49]. Likewise, in *C. elegans* germline specific SUN-domain protein SUN1/Matefin interacts with KASH-motif bearing ZYG-12 to form a SUN-KASH-complex that, in the worm, is essential for telomere attachment and congregation to the centrosome [19, 50]. In mammals, with Sun1 and Sun2 two different members of the Sun-domain

family have recently been identified to participate in forming the meiotic telomere attachment complex [20, 51]. In somatic cells, both proteins are uniformly distributed throughout the NE, whereas in meiotic cells their localization is restricted exclusively to the sites of telomere attachment (fig. 1C, D). At these sites, Sun2 takes part in forming the filaments that were seen to interconnect telomeres with cytoplasmic structures [51]. The imperative necessity of a functional SUN-KASH-complex for meiotic telomere attachment and bouquet formation has been demonstrated by various genetic approaches performed in different species. Mutations in either Sun-domain proteins or their respective KASH-domain partners have dramatic consequences for meiotic progression. In an *S. pombe* mutant strain lacking Kms1, telomeres fail to cluster efficiently at the SPB and homolog pairing appears to be significantly impaired [13]. In the worm, SUN1/Matefin carrying a missense mutation in the SUN-domain that causes a disruption of the SUN-KASH-complex accounts for massive failures in homolog pairing and recombination [19]. Likewise, disruption of Sun1 in mice prevents faithful telomere attachment and bouquet formation and severely interferes with chromosome pairing, synapsis and recombination [20].

Attachment of telomeres to the NE via the nucleoplasmic domain of SUN-domain proteins apparently depends on the presence of additional, however meiosis-specific adapter molecules. In fission yeast, interaction between Sad1 and telomeres is mediated by a complex of two proteins, Bqt1 and Bqt2. Thereby, Sad1 directly interacts with Bqt1, which on its part binds telomere-associated Rap1, but only in the presence of Bqt2, hence generating a tight structural linkage between telomeres and the NE [47, 52, 53]. In the absence of either Bqt1 or Bqt2 telomeres do not colocalize with Sad1, moreover they fail to cluster and remain dispersed in multiple locations at the nuclear periphery [47, 52]. A similar function in interconnecting telomeres with the NE docking sites might be occupied by Ndj1 in budding yeast. Ndj1 is a telomere binding protein that was found to interact with Mps3 [18, 54]. Deletion of Ndj1 severely impedes telomere association with the NE so that part of the telomeres are found residing in the nuclear interior. As a consequence, telomeres do not cluster and chromosomes fail to pair and recombine properly [16, 18, 54, 55]. Remarkably, the NE of mammalian somatic cells contains both Sun1 and Sun2, but in the non-meiotic condition telomeres are not bound to the nuclear envelope and remain more or less internal. Thus, in mammals for meiosis specific translocation of telomeres to the NE and their anchorage to SUN-domain proteins a similar adapter mechanism is to be expected. However, functional orthologs of fission yeast Bqt1 and Bqt2 or budding yeast Ndj1 have not yet been identified in higher eukaryotes and finding these orthologs remains an important challenge for the near future.

Meiotic telomere attachment and their directed movements within the NE require, at least in mammals, a more general reorganization of the NE. This in particular concerns the nuclear lamina, i.e. the structural element of the NE that is critically involved in many fundamental cellular and developmental processes [for recent reviews see 56, 57]. Mammalian meiotic cells are characterized by the absence of three of the four

lamin isoforms that are typically expressed in differentiated somatic cells [58]. Instead, during meiotic prophase I they express a novel lamin, lamin C2, a short meiosis-specific isoform of the lamin A gene [59, 60]. Interestingly, and in contrast to the situation of lamins in somatic cells that are distributed in a continuous ring-like pattern, lamin C2 is distributed in form of discontinuous domains at the nuclear periphery, a phenomenon that most likely can be assigned to the missing N-terminal domain [61, 62, unpublished data]. Moreover and most remarkable, lamin C2 appears highly enriched at the sites of telomere attachment. Hence, it was proposed that lamin C2 might lead to a local flexibilization of the NE at the attachment sites that in turn enables movement of telomeres and therefore supports pairing, recombination and synapsis [61, 62]. Accordingly, in male mice lacking A-type lamins massive failures during the process of pairing and synapsis of the homologs have been observed [63].

Seeking for the Motor

How do chromosomal ends move on the plane of the NE and which are the driving forces for bouquet formation? This long standing and very central question is a matter of an intense, but somehow also controversial debate. The ultrastructural appearance that shows filaments arising from the telomere attachment sites then crossing the nuclear membranes and radiating into the cytoplasm led to the suggestion that telomere movements are driven by forces most likely acting from the cytoplasm. This hypothesis was clearly supported in the past years by the molecular uncovering of the evolutionarily highly conserved SUN-KASH complex that acts as a molecular linker connecting telomeres to cytoplasmic structures (fig. 2; see above). Hence, from the present data the presence of a motor operating from the outside appears more than overt and is generally accepted. However, a contribution of active forces acting from the nuclear interior cannot yet be excluded.

In case of fission yeast a cytoplasmic microtubule (MT) dependency of telomere movements is deemed to be proven. Via Bqt1/2 meiotic telomeres attach to the Sad1-Kms1 membrane spanning complex that directly connects to the SPB. Recently, it was shown that Kms1, which represents the ONM partner of the complex, interacts at the cytoplasmic side with dynein light chain [64], suggesting that a microtubule motor operating from the cytoplasm might be involved in the directed telomere and chromosomal movements. Consistently, absence of dynein motor proteins Dlc1 or Dhc1, respectively, severely interferes with efficient homolog pairing and recombination [17, 65, 66]. These findings are in clear contrast to the situation in budding yeast, where an MT dependent movement could be excluded. Instead, bouquet formation in *S. cerevisiae* appears to rely solely on actin [16, 67]. In rye, bouquet formation is inhibited by MT destabilizing drugs, but does not require cytoplasmic microtubules. Due to this discrepancy, it was suggested that a tubulin-like protein may be somehow involved in directed telomere movement in plants [68–71]. Meiotic progression in

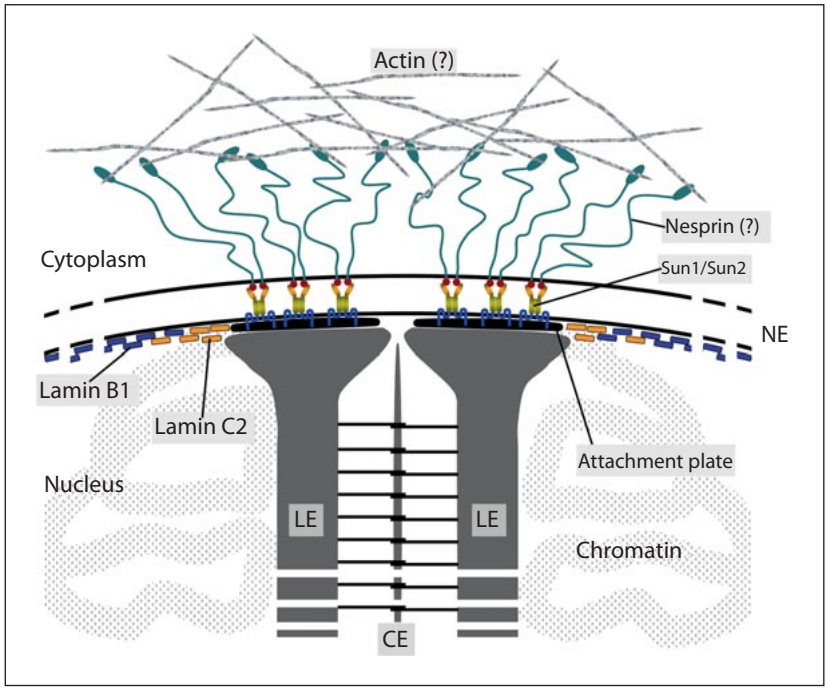

Fig. 2. Model for the organization of the NE at the attachment sites of meiotic chromosomes. In mammalian meiotic cells Sun1 and Sun2 are exclusively located at the attachment plates. There, they take part in tethering meiotic telomeres to the NE [20, 51]. Within the perinuclear space their C-terminal SUN-domain binds to the KASH domain of nesprins, thus forming a stable fibrillar complex bridging both nuclear membranes. Because nesprins were shown to interact with cytoplasmic actin via their actin binding domain [72–75], the formation of such a complex would link telomeres to the actin cytoskeleton. This model is consistent with recent findings that identified an actin-dependent mechanism for bouquet formation in budding yeast [16, 67]. LE, lateral element; CE, central element.

mammalian spermatogenesis appears to be sensitive to MT destabilizing drugs as well, but it is currently not clear, whether the drugs directly affect telomere dynamics or the more general nuclear oscillations that are observed during early meiosis and proposed to be crucial for germ cell viability [4]. However, the fact that mammals use SUN-domain proteins Sun1 and Sun2 as the central NE docking sites for meiotic telomeres [20, 51] would favor an actin dependent mechanism. Both Sun1 and Sun2 were shown to interact with KASH-domain containing nesprins that in turn were found to connect to the actin cytoskeleton in somatic cells [72–75]. Given that this connection is established in meiosis as well, similar to *S. cerevisiae* actin could represent the primary driving force for directed meiotic telomere movements that trail telomeres into bouquet configuration, hence supporting homolog recognition and pairing [see fig. 2; 51]. However, up to the present it is not known, whether nesprins are expressed during meiotic prophase, thus this very important question remains to be investigated in the future.

In summary, meiotic telomere attachment and bouquet formation does not only correlate between the species on the cytological level, but it appears evolutionarily highly conserved on the molecular level as well. It depends on a general mechanism with SUN-KASH complexes playing a very central role in that they link telomeres to the cytoskeleton. Despite this conservation, a general conserved motor that drives telomere congregation has not been identified so far, rather the driving forces appear to vary between the different species, with a species specific preference for actin and/or MTs. However, in this matter we are just at the beginning and thus unraveling the molecular motors remains a very important and interesting quest for the future.

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Cohesin Complexes and Sister Chromatid Cohesion in Mammalian Meiosis

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Abstract

Maintenance and precise regulation of sister chromatid cohesion is essential to ensure correct attachment of chromosomes to the spindle, thus preserving genome integrity by correct chromosome segregation. Errors in these processes often lead to aneuploidy, frequently implicated in cell death and/or tumor development. The so-called cohesin complexes are essential in sister chromatid cohesion during both mitosis and meiosis; they are responsible for maintaining sister chromatids together physically until their segregation during the metaphase/anaphase transition. The recent identification of new molecules involved in the control of sister chromatid cohesion, and the characterization of mouse loss-of-function models, have improved our understanding of the variety of cohesin complexes and their chromatin binding and removal regulation. This review will focus basically on the distribution and function of cohesin complexes during mammalian meiosis.
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During cell division, DNA replication produces a pair of sister chromatids with identical genetic content that must be maintained together throughout the G2 phase. The loss of sister chromatid cohesion at the metaphase/anaphase transition is probably one of the most exciting and controlled processes in the life of the cell, since inaccuracy in the regulation of this step frequently has disastrous consequences for daughter cells. Failure of the mechanisms that maintain correct chromosome segregation during meiosis has catastrophic effects that promote infertility and miscarriage. Infertility is estimated to affect 15% of couples, and a significant proportion of this is due to meiosis defects. Aneuploidy is a further clinical manifestation of problems in meiosis, which are more common in females.

Sister chromatid cohesion is mediated by a multi-protein cohesin complex, which was first characterized in *Saccharomyces* and *Xenopus* [1, 2]. The cohesin complex has four core subunits conserved throughout evolution: two structural maintenance of

Table 1. Subunits of the cohesin complexes

	S. cerevisiae	*S. pombe*	*C. elegans*	*Drosophila*	*Xenopus*	Mammals
Structural maintenance of chromosomes (SMC)	Smc1 Smc3	Psm1 Psm3	Him-1 Smc3	Smc1 Smc3	XSMC1 XSMC3	SMC1α SMC3
Meiosis-specific						SMC1β
Kleisin α[a]	Mcd1/Scc1	Rad21	Coh-2/Scc1	DRad21	XRAD21	RAD21
Meiosis-specific	Rec8	Rec8	Rec8		XREC8	REC8
Stromalin/HEAT repeat domain	Scc3/IRR1	Psc3	Scc3	DSA1	XSA1, XSA2	STAG1, STAG2
Meiosis-specific		Rec11		MNM/DSA2		STAG3

[a]From the Greek word for 'closure' [106].

chromosomes family proteins (SMC1 and SMC3), one kleisin α subunit (SCC1/RAD21), and a HEAT-repeat domain protein (SCC3/SA/STAG) (table 1). Higher eukaryotes have two mitotic SA/STAG family members, SA1/STAG1 and SA2/STAG2 [3], which do not coexist and are present in different cohesin complexes in *Xenopus* and man [4]. These complexes form a ring-like structure and mediate cohesion by embracing chromatin fibers from the two sister chromatids [5, 6] (fig. 1C). In addition to their canonical role as chromosome 'glue' during cell division, cohesins are involved in other aspects of post-replicative double-strand break repair and gene expression in interphase [for reviews see 7 and 8].

In *Saccharomyces cerevisiae* mitosis, the cohesin complexes are loaded near G1 to S phase, but cohesins bind to chromatin in telophase in most organisms studied [9]. Establishment of cohesion also depends on the Scc2/Scc4 adherin complex [10, 11] and on the Eco1/Ctf7p acetyltransferase [12–14]. Cohesin complexes are released from chromatin at the metaphase/anaphase transition after separase, a specific cysteine protease, cleaves the SCC1 subunit of the cohesin complex, destabilizing cohesion and allowing chromatid segregation [15]. Before anaphase, separase remains inactivated by binding to its specific inhibitor securin [16–19], and in vertebrates also by CDK1-mediated phosphorylation [20]. In metazoa, dissociation of cohesin complexes from chromatin proceeds in a two-step manner. In a first step, the bulk of cohesin complexes is removed from chromosome arms during prophase by a separase-independent pathway [21], in which phosphorylation of the SA2/STAG2 subunit by Aurora B and Polo-like kinases triggers the removal of arm cohesins [22]. A cohesin complex population remains essentially at centromeres until the chromosomes are correctly bioriented and the spindle assembly checkpoint is satisfied in metaphase. Activation of the anaphase promoting complex/cyclosome (APC/C) leads to ubiquitination of securin; this allows

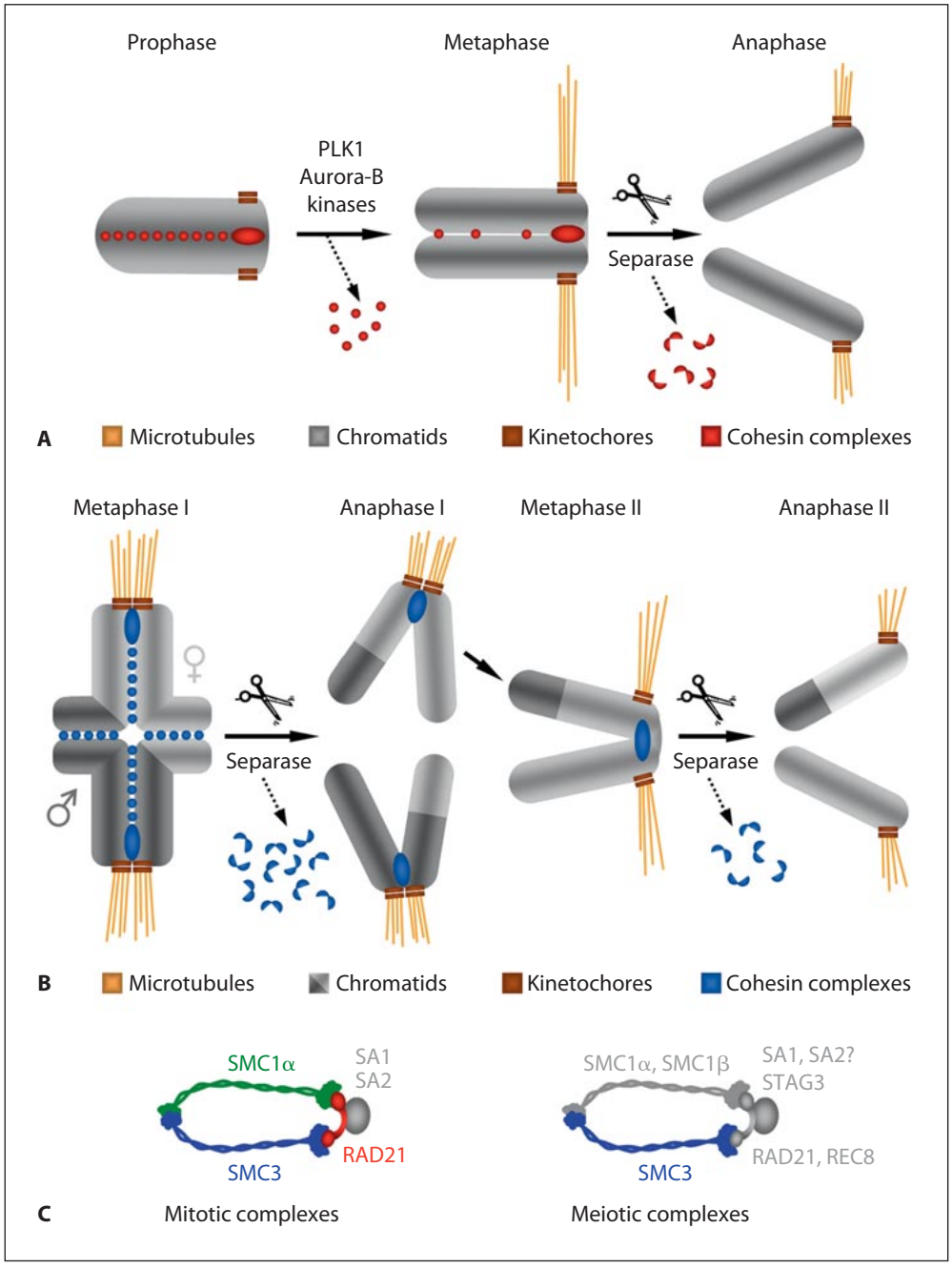

Fig. 1. Chromosome segregation in mammalian mitosis and meiosis, and cohesin complexes. **A** Mitosis. In prophase chromosomes, that still do not show two resolved sister chromatids, the cohesin complexes are located along the middle region of the arm and at the inner centromere domain. During the prophase/metaphase transition the kinases Aurora-B and/or Polo-like kinase 1 (PLK1) phosphorylate the SA2 subunit of most cohesin complexes at the arm and promote their loss from chromosomes, that now show two resolved chromatids. The few cohesin complexes remaining at the arm and those present at centromeres are cleaved by separase once all chromosomes are

cleavage of RAD21 from centromeric cohesin complexes by separase, triggering the onset of anaphase [23] (fig. 1A). Besides this general scheme, it was recently reported that separase is required for cleavage of the few cohesin complexes remaining at the arms of metaphase chromosomes in human cells [24] (fig. 1A).

Meiosis is characterized by a single round of DNA replication, followed by two rounds of chromosome segregation, to yield haploid gametes from diploid germ cells. During meiosis I, the cohesin complexes at arms are removed during the metaphase I/anaphase I transition to allow segregation of recombined homologs to opposite poles. The centromeric cohesin complexes remain associated to chromosomes until the onset of anaphase II [9, 25, 26] (fig. 1B). This is necessary to prevent premature separation of sister chromatids and thus, aneuploidy in the resulting gametes. Loss of arm and centromere cohesion in meiosis I and II, respectively, takes place by a separase-dependent mechanism in most models studied [9], with the exception of some results in *Xenopus* oocytes [27, 28]. This chapter reviews current knowledge of the distribution and dynamics of different cohesin complexes during prophase I and both mammalian meiotic divisions, as well as of the molecules that regulate their association/dissociation from chromatin.

Meiotic Cohesin Complexes

In somatic cells, the cohesin complex consists of the four canonical subunits mentioned above, but in germ cells, distinct meiosis-specific subunits have been characterized in various organisms (table 1). In mammals, the meiotic paralogs of SMC1, SCC1/RAD21 and SA/STAG1/2 are thus SMC1β [29], REC8 [30, 31] and STAG3 [32, 33], respectively. Whereas these three cohesin subunits are expressed only in germ cells, and are not involved in mitotic chromosome segregation, canonical SMC1α (to distinguish from the meiosis-specific subunit), RAD21 [34–36] and STAG2 [34] are

bioriented at the metaphase plate. The chromosome depicted is telocentric. **B** Meiosis. In metaphase I bivalents, the cohesin complexes are located at the interchromatid domain along the arms, and at the inner centromere domain below the closely associated sister kinetochores. At the onset of anaphase I, only the cohesin complexes at the arms are cleaved by separase to allow the segregation of recombined homologs to opposite poles. Each chromosome shows two chromatids that are only joined at the centromere where cohesin complexes persist. In metaphase II chromosomes, sister kinetochores attach to microtubules from opposite poles, and cohesin complexes are found at the inner centromere domain. During the metaphase II/anaphase II transition separase cleaves those remaining cohesin complexes to trigger chromatid segregation. Chromosomes depicted are telocentric, and the bivalent shows a single interstitial chiasma. **C** Mammalian cohesin complexes. During mitosis, the cohesin complex is formed by SMC1α, SMC3, RAD21 and either SA1 or SA2. During meiosis, there are several cohesin complexes with either SMC1α or SMC1β, SMC3, RAD21 or REC8, and STAG3. The presence of SA1 and SA2 could increase the variety of complexes. We used SA for SA1 and SA2 and STAG for STAG3 in the cohesin complexes in order to maintain the most widespread nomenclature for the SA/STAG members in the literature.

reported to be implicated in meiotic chromosome dynamics. Results of immunofluorescence and immunoprecipitation studies in meiocytes from several rodent models showed good evidence of participation of different cohesin complexes during mammalian meiosis. Characterization of distinct meiotic cohesin complexes containing either REC8 or RAD21 as kleisin α subunits, which have distinct localization patterns and dynamics, as well as the simultaneous presence of SMC1α- and SMC1β-containing complexes and the presence of STAG2 and STAG3 in mammalian meiosis suggest a large variety of putative cohesin complexes formed by combinations of cohesin subunits. Based on these results, there are thought to be at least four or five distinct cohesin complexes in mammalian meiosis [reviewed in 37]. These complexes show differences in spatio-temporal distribution throughout the meiotic divisions, and deficiency in individual cohesin subunits results in distinct phenotypes in mouse models. These results strongly indicate specific functions for each cohesin complex in the maintenance of genome structure during chromosome segregation in meiosis.

Rad21 is involved in meiosis in both the fission yeast *Schizosaccharomyces pombe* [38] and the budding yeast *S. cerevisiae* [39]. In addition, fission yeast has two Scc3 homologs, called Psc3 and the meiosis-specific Rec11. Both cohesin subunits are able to interact with Rec8, forming two cohesin complexes in fission yeast meiosis. The Rec8-Rec11 complex is located along the chromosome arms, whereas the Rec8-Psc3 complex is found in the vicinity of centromeres, and selective inactivation of Psc3 or Rec11 allows distinction of cohesin complex functions along the arms and at centromeric regions [40].

Synaptonemal Complex Proteins

The division of the germinal cells, meiosis, shows important differences with mitosis in regard to the way genetic material is distributed to daughter cells. During prophase of the first meiotic division (prophase I), the homologs, each with a pair of cohered sister chromatids, pair by still-unknown mechanisms, and in most organisms give rise to a meiosis-specific nucleo-proteic structure, the synaptonemal complex (SC) [41]. This structure represents an essential difference with mitosis and supports meiotic recombination, the source of genetic variability. Prophase I is the longest meiotic step, and has historically been subdivided into stages based on early cytological studies. During leptotene, the axial elements (AE) form along each chromosome; in zygotene, the homologs with their AE, begin to pair, and a new structure, the central element (CE), emerges between the AE (now termed lateral elements; LE). In pachytene, the homologs have synapsed all along their length and the SC is completely formed, and recombination and DNA repair take place. The product of the recombination between non-sister chromatids causes chiasmata formation. Disassembly of the SC and homolog desynapsis characterize the onset of diplotene. The homologs remain connected at chiasmata in diplotene, and cohesion between sister chromatids prevents

premature incorrect homolog segregation. In mammals, two proteins, SYCP2 [42] and SYCP3 [43], have been characterized as the main structural protein components of AE/LE during meiotic prophase I. The transverse filaments (TF) that form the CE are composed essentially by SYCP1 [44]. In addition to these and other more recently characterized SC proteins (see next sections), cohesin complexes are also implicated in SC formation and maintenance.

Cohesion during Mammalian Prophase I

Chronologically, the first report of cohesins in mammalian meiosis showed that, in spread rat spermatocytes, SMC1α and SMC3 subunits appeared as dots in a bead-like arrangement along asynapsed AE in late zygotene, and along synapsed and desynapsed LE at pachytene and diplotene, respectively [45]. By co-immunoprecipitation experiments, this study also showed that these cohesin subunits interacted with the LE proteins SYCP2 and SYCP3. It was later corroborated that SMC1α is lost from desynapsed LE at diplotene and is not detected in diakinesis and metaphase I bivalents [29]. In contrast, SMC3 persists at desynapsed LE during diplotene, but is then progressively lost from LE, to accumulate at centromeres by diakinesis [29]. This same group reported a new SMC1 meiosis-specific paralog termed SMC1β. This cohesin subunit was detected along the asynapsed AE at leptotene and zygotene, and at synapsed and desynapsed LE from zygotene to diplotene. As for SMC3, it was proposed that SMC1β is lost from desynapsed LE at late diplotene and accumulates at centromeres during diakinesis [29]. The meiosis-specific cohesin subunit STAG3 was then detected on squashed mouse spermatocytes along AE/LE from leptotene to diplotene [33]. In contrast to the reported accumulation of SMC1β and SMC3 at centromeres during diakinesis, STAG3 persists as patches along the contact surface between sister chromatids, at the so-called interchromatid domain [46], in diakinesis bivalents [33]. These authors proposed that although there is partial loss of STAG3 from chromosome arms during diakinesis, this cohesin subunit is maintained at chromosome arms, and also at centromeres, until metaphase I [33]. Some years later, distribution of the meiosis-specific subunit REC8 was analyzed in two studies on spread rat [47] and mouse [48] spermatocytes. REC8, like SMC1β, SMC3 and STAG3, is distributed along asynapsed, synapsed and desynapsed AE/LE during all stages of prophase I. As previously reported for STAG3 [33], REC8 was also found at the interchromatid domain along chromosome arms and at centromere, in diakinesis and metaphase I bivalents [47, 48]. Immunoprecipitation experiments showed that REC8 interacts with SMC1β but not with SMC1α [48].

Although it was proposed that REC8 replaces RAD21 during mammalian meiosis, RAD21 was detected in mouse spermatocytes [34], and two independent studies demonstrated that RAD21 is present during prophase I on both squashed [35] and spread [36] mouse spermatocytes. RAD21, like all previously described subunits,

appears at AE/LE from leptotene to diplotene [35, 36]. During late diplotene, however, RAD21 appears not only along desynapsed LE, but also at accumulations along them that colocalize with SYCP3. By diakinesis, these RAD21/SYCP3 accumulations seem to leave the chromosome arms, to appear as large agglomerates in the nucleoplasm. Concomitantly, there was a notable increase in dissociated RAD21 in the nucleoplasm and an accumulation at centromeres [35]. These observations were the first indication that RAD21 is partially released from LE from late diplotene to diakinesis. RAD21 release during these stages is not observed after spermatocyte spreading [36]. The squash methodology is thus a valuable tool that allows accurate analysis of cohesin subunit release during diakinesis. The diakinesis stage has been overlooked in the past, since the number of spermatocytes in this stage is very low. The diakinesis/metaphase I transition is nonetheless of outstanding relevance, since most spindle-assembly checkpoint proteins associate to centromeres [49], condensin I complexes associate to chromosomes [50], and many cohesin complexes are released from chromosome arms. The molecular mechanisms that regulate the differential dissociation of distinct cohesin complexes from chromosome arms during diakinesis are not known. This partial release of cohesin appears to be separase-independent, and suggests the phosphorylation of some cohesin subunits, as occurs during vertebrate mitotic prophase [21, 22]. Likewise, the mechanisms that regulate the accumulation of certain cohesin complexes at centromeres during diakinesis remain a mystery. Although a fraction of mammalian STAG2 is reported to be present in some meiotic stages in mouse spermatocytes [34], further studies are needed to clarify STAG1 and/or STAG2 involvement in meiotic cohesin complexes.

STAG3, REC8, SMC1β and RAD21 are also expressed in mammalian fetal oocytes, and their dynamics during early prophase I is similar to that in spermatocytes [51]. Total loss of cohesin signals, as well as of SYCP2 and SYCP3, is nonetheless observed in both human and mouse oocytes as they progress through dictyate arrest [51, 52]; they are again detected on bivalents in metaphase I oocytes [52–54]. To date, there are no studies that follow cohesin dynamics during the dictyate/metaphase I transition in mammalian oocytes. It thus remains unknown whether cohesin complexes persist on chromosomes during this transition but are not reactive with specific antibodies, or whether they are destroyed, newly synthesized and loaded onto chromosomes. Studies of cohesion on *Smc1β*-deficient oocytes [55] and on oocytes from senescence-accelerated mice in which REC8, STAG3 and SMC1β were greatly reduced [56], suggest that defective cohesin levels are associated with age-related nondisjunction in oocytes.

Taking all these studies into account, at least three types of cohesin complexes are present during mammalian prophase I [26]. The first, formed by SMC1α/SMC3/REC8/STAG3, would be present along AE/LE only to diplotene. Two additional complexes, formed by SMC1β/SMC3/REC8/STAG3 (the canonical meiotic complex) and SMC1β/SMC3/RAD21/STAG1 or STAG2, would be present throughout prophase I. If SA/STAG1 and SA/STAG2 expression during mammalian meiosis is demonstrated,

the number of cohesin complexes might increase, since these subunits could interact with complexes containing either RAD21 or REC8.

Cohesin Axes and Axial Elements

The appearance of AE at leptotene, marked by SYCP2 and SYCP3 expression, is parallel to the detection of cohesin subunits [35, 36]. REC8 is reported to appear in the nuclei before premeiotic S phase, however, and to form AE-like structures from premeiotic S phase onward, while the cohesin subunits SMC1β and SMC3, as well as SYCP2 and SYCP3, are loaded onto these REC8-labeled AE shortly afterwards [47]. These results suggested that the cohesin subunits are loaded sequentially onto chromosomes. Likewise, it was reported that REC8 and STAG3 can be detected earlier than SYCP3 in mouse and human fetal oocytes [51]. Based on these results, it was proposed that a cohesin axis is pre-formed along homologs and acts as the organizing framework for subsequent AE/LE anchorage and formation [51]. This hypothesis is reinforced by the fact that the cohesin axis is organized even in the absence of LE and is able to recruit several TF components to form a CE-like structure in *Sycp3*- and *Sycp2*-deficient mice [57–59].

It is interesting to note that in grasshopper meiosis, the SMC3 ortholog is detected as early as leptotene on unsynapsed AE in autosomes, and on the single AE of the unsynapsed X chromosome, whereas the non-SMC subunit orthologs SA1 and RAD21 are not detected until zygotene over synapsed regions of bivalents [60]. This temporal separation in the assembly of distinct cohesin subunits is compatible with a model in which the sequential loading of various cohesin complexes could reinforce and stabilize the bivalent structure during prophase I [60]. Differential loading of cohesin subunits has been indirectly suggested in *Caenorhabditis elegans*. In addition to the above-mentioned detection of REC8 in the mammalian pre-meiotic S phase, the depletion of *C. elegans* TIM-1 (the ortholog to the *Drosophila* clock protein TIMELESS) prevents assembly of non-SMC subunits, but not the loading of SMC components [61].

Cohesion during Mammalian Meiotic Divisions

During the metaphase I/anaphase I transition, cohesion is lost between sister chromatid arms, allowing homolog segregation. The cohesion that persists, essentially at the centromeres as in mitosis, is released during the metaphase II/anaphase II transition; the chromatids are segregated and four haploid cells are generated from a single progenitor cell.

In metaphase I mouse bivalents, both in spermatocytes and oocytes, STAG3 is detected as a series of bright patches at the interchromatid domain (fig. 2) along the arms, except at chiasma sites [33, 52] (fig. 3D). STAG3 is also present at the inner

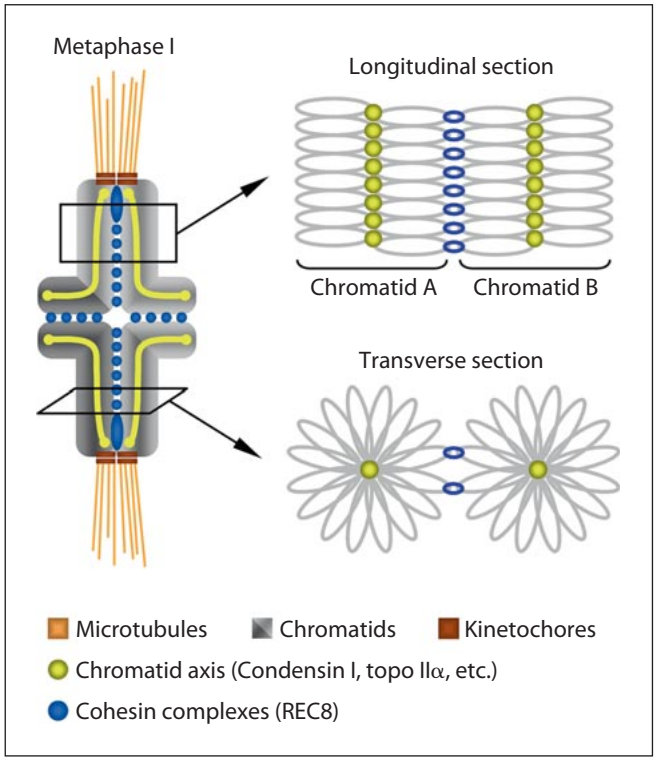

Fig. 2. Schematic representation of a mouse metaphase I bivalent showing the relative distributions of condensin I and REC8-containing cohesin complexes. Chromosomes are telocentric, and the metaphase I bivalent shows a single interstitial chiasma. The condensin I complexes delineate a fuzzy axis inside each chromatid, with prominent accumulations at their proximal and distal ends. The two proximal condensin I accumulations appear below the closely associated sister kinetochores. REC8-containing cohesin complexes are depicted as patches at the interchromatid domain and at the inner centromere region. A hypothetical model is presented accounting for the distribution of condensin I and REC8-containing cohesin complexes in relation to radial chromatin loops. The longitudinal and transverse sections of the arms correspond to areas indicated on the bivalent.

centromere domain just below the closely associated sister kinetochores [33]. The same kind of labeling has also been reported for the cohesin subunit REC8 in metaphase I bivalents [47, 48, 52–54, 62] (fig. 3A). Although it was initially reported in metaphase I bivalents that SMC3 was only concentrated at centromeres and was absent from chromosome arms [29, 47], our unpublished results show that this subunit has the same distribution as STAG3 and REC8 (fig. 3C). In contrast, RAD21 distribution in metaphase I bivalents is distinct. This subunit, which colocalizes completely with SYCP3 and SYCP2 during meiosis [35, 36], accumulates preferentially at the inner centromere domain, where it shows a 'double cornet'-like arrangement just below and surrounding the closely associated sister kinetochores [35]. Moreover, RAD21 is detected as a few faint patches at the interchromatid domain [35] (fig. 3B). This labeling pattern is also reported for SMC1β, although the precise three-dimensional arrangement at the inner

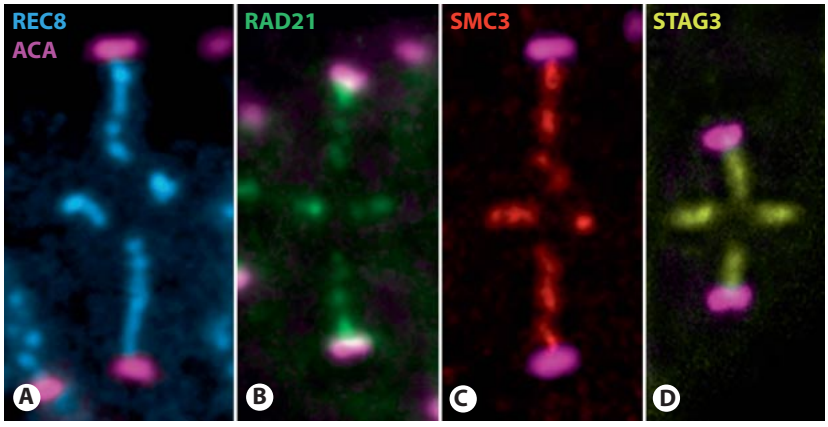

Fig. 3. Distribution of the cohesin subunits REC8, RAD21, SMC3 and STAG3 in mouse metaphase I bivalents. The subunits REC8, SMC3 and STAG3 are preferentially located at the interchromatid domain as bright patches, although they are also present at the inner centromere domain below the closely associated sister kinetochores. By contrast, RAD21 is preferentially located at the inner centromere domain where it shows a T-shaped distribution, although it is also present at the interchromatid domain as few faint patches. The telocentric bivalents show a single interstitial chiasma. The labeling for these different cohesin subunits always interrupts at the chiasma site. Images have been pseudocolored.

centromere domain has not been studied [52]. Together, these results suggest the existence of different cohesin complexes in mammalian metaphase I bivalents (fig. 4). Two complexes would be present at the interchromatid domain along chromosome arms, one formed by SMC1β/SMC3/REC8/STAG3 (the canonical meiotic complex), and another formed by SMC1β/SMC3/RAD21/STAG3. The identity of the centromere complexes is more complicated since RAD21, and apparently also SMC1β, occupy the entire inner centromere domain showing a 'double cornet'-like three dimensional arrangement, whereas STAG3, SMC3 and REC8 are present only at a vertical subdomain within the inner centromere domain (fig. 3). If correct, this would imply that the RAD21/SMC1β-containing complexes at the two rings do neither contain SMC3 nor STAG3 (fig. 4, complex 5). Further study is clearly needed to determine the identity of the distinct centromere cohesin complexes. The presence of SMC1α, STAG1 and STAG2 in metaphase I bivalents needs to be confirmed conclusively or refuted. Moreover, the greater SMC3 signal (fig. 3) compared to SMC1β [52] at the interchromatid domain in arms suggests a possible sixth complex, formed by an SMC3 homodimer, and STAG3 and REC8 (fig. 4, complex 6).

The precise distribution of cohesin subunits from anaphase I to metaphase II is mostly unknown. During anaphase I, after release of arm cohesion, STAG3 [33], REC8 [47, 48, 53, 62], SMC3 [47], SMC1β [47], and RAD21 [35, 36, 62] persist at centromeres, although they are distributed differently. Their dynamics during telophase I and interkinesis is nonetheless uncertain. Interkinesis, the interphase between meiosis I and II, has been recurrently ignored, probably because there are no morphological criteria to distinguish these nuclei after spermatocyte spreading. Interkinesis is thus a

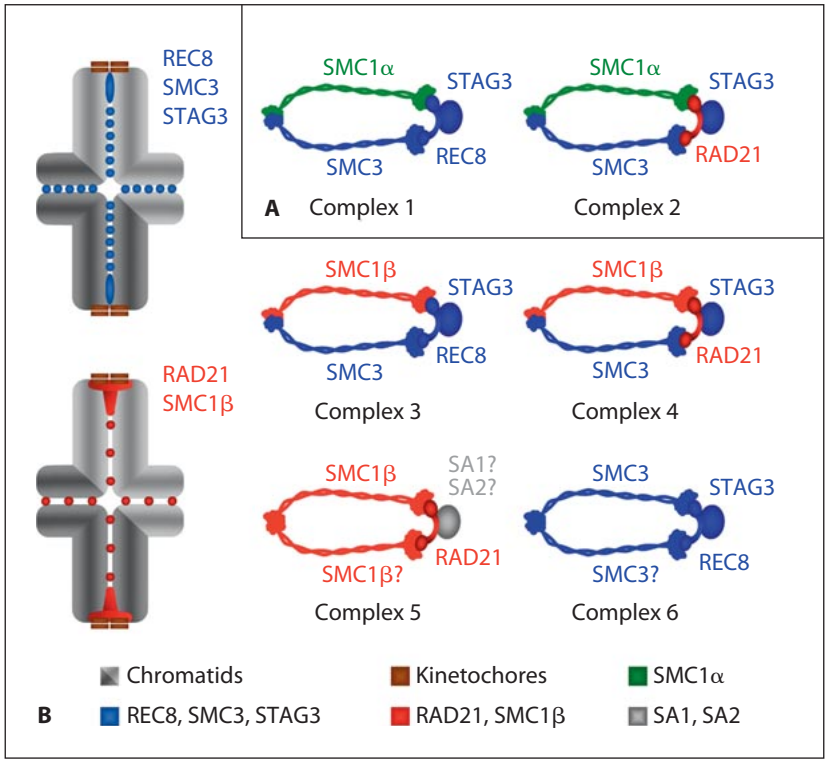

Fig. 4. Putative cohesin complexes during mammalian meiosis. Based on the cytological localization of cohesin subunits, from leptotene up to diplotene, and along AEs/LEs, different SMC1α- and SMC1β-containing complexes (complexes 1, 2, 3 and 4) could be present. **A** Complex 1 formed by SMC1α, SMC3, REC8 and STAG3 has been demonstrated. However, there exists the possibility of another SMC1α-based complex (complex 2) formed by SMC1α, SMC3, STAG3 and RAD21 instead of REC8. These both SMC1α-containing complexes would be not present after diplotene. **B** In metaphase I bivalents, the 'canonical' meiotic complexes (complex 3) formed by SMC1β, SMC3, REC8 and STAG3 are present at the interchromatid domain, and at the inner centromere domain showing a vertical arrangement in relation to the closely associated sister kinetochores. Another complex (complex 4) would be formed by SMC1β, SMC3, STAG3 and RAD21 instead of REC8, and as complexes of type 3, are also located at the interchromatid domain, showing a vertical arrangement in relation to sister kinetochores at the inner centromere domain. RAD21, and apparently also SMC1β, show a T-like arrangement at the inner domain of metaphase I centromeres. However, SMC3, REC8 and STAG3 are not present at the horizontal region of the T-labeled structures by RAD21 antibodies. Consequently, we hypothesize the presence of a new cohesin complex (complex 5) formed by a homodimer SMC1β/SMC1β, RAD21 and SA1 or SA2, although the presence of these two last subunits has still not been accurately demonstrated. Finally, we could not discard the possibility of a sixth complex formed by a homodimer of SMC3, STAG3 and REC8 at the interchromatid domain based on the immunofluorescence detection results (see text for details).

'black box' in mammalian meiosis, since it is difficult even in histological sections to differentiate interkinetic spermatocytes from early round spermatids.

Using the squash technique, we found that RAD21, which colocalizes with SYCP3 and SYCP2, changes its distribution from a 'double cornet' at anaphase I centromeres

to a bar in between sister kinetochores or displaced from them at telophase I centromeres (fig. 5A). Since this RAD21 redistribution is concomitant with the separation between sister kinetochores observed at telophase I, it has been suggested that RAD21 could regulate their association, or sister kinetochore cohesion, an essential phenomenon that allows kinetochore monopolar attachment and biorientation of bivalents during prometaphase I [35]. The RAD21 bars detected at telophase I centromeres also appear during interkinesis at heterochromatic chromocenters, representing closely associated centromeres, but disappear at prophase II and are no longer detected at metaphase II [35] (fig. 5A, 6). It was also reported that REC8 and STAG3 are apparently lost from centromeres during telophase I and are no longer detected in interkinesis nuclei [33, 62] (fig. 5B). Nonetheless, there are no data on SMC1β and SMC3 distribution during interkinesis and prophase II. Accordingly, the putative degradation of some cohesin complexes during telophase I, and their synthesis and reloading onto centromeres during interkinesis and/or prophase II during mammalian meiosis remains an open question.

The presence and precise distribution of cohesin subunits at mammalian metaphase II centromeres is controversial. Initial studies in mouse and rat spread spermatocytes indicated that SMC1β [29] and RAD21 [36], as well as the LE protein SYCP3 [29, 36], appeared as elongated aggregates connecting the separated sister kinetochores at metaphase II centromeres. In contrast, RAD21 and SYCP3 were not detected at centromeres on metaphase II squashed spermatocytes [35, 62]. In our opinion, the spermatocytes described as metaphase II after spreading in fact correspond to telophase I and early interkinesis nuclei. In squashed spermatocytes, RAD21 and SYCP3 appear as bars or elongated aggregates between separated sister kinetochores only during these stages, and not during metaphase II [35]. The squash technique does not disturb chromosome condensation and nuclear envelope integrity as occurs with the spreading method, and simple DAPI staining then allows unequivocal differentiation of all meiotic stages. We cannot exclude the presence of cohesin complexes at metaphase II centromeres, however, since as the old adage says 'absence of evidence is not evidence of absence'. Indeed, it is possible that the antibodies used to detect cohesin subunits do not reveal small quantities of them at metaphase II centromeres after spermatocyte squashing. Recent results using mouse oocytes indicate that REC8 appears as small patches at the inner domain of metaphase II centromeres, and not as elongated bars connecting sister kinetochores, as proposed [53, 54, 63]. Thus, cohesin complexes containing REC8 could regulate centromeric cohesion until the second meiotic division (fig. 6), as occurs in yeasts and *C. elegans*.

Cohesin- and/or SC Protein-Deficient Mice

The characterization of mice deficient in either meiosis-specific cohesin subunits and/or in SC proteins has helped understand the function of these proteins during in

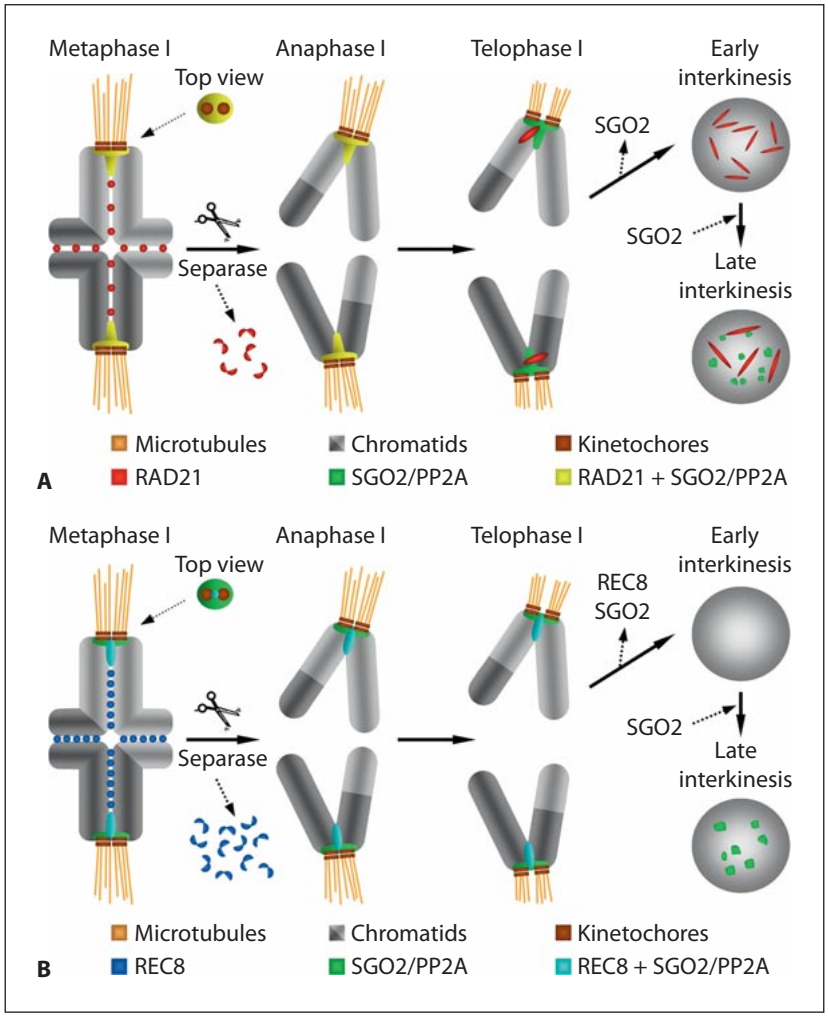

Fig. 5. Schematic representation summarizing current data about of distributions of RAD21 (**A**) and REC8 (**B**) in relation to that of SGO2/PP2A complex from metaphase I up to late interkinesis in mouse. In metaphase I bivalents and segregating anaphase I chromosomes, SGO2 and RAD21 colocalize as a 'double cornet'-like 3D structure at the inner centromeric domain below the closely associated sister kinetochores, when centromeres are side-viewed. By contrast, REC8 only colocalizes with SGO2 and RAD21 in the vertical region of the 'double cornet' structure. In top-viewed metaphase I centromeres, SGO2 and RAD21 appear as two closely associated rings, while REC8 is observed as a round spot at the region of contact between the rings. During the metaphase I/anaphase I transition, arm cohesin complexes with either RAD21 or REC8 are cleaved by separase, but centromeric complexes are protected by SGO2/PP2A. During telophase I, SGO2 and REC8 preserve their localization, but RAD21 changes its distribution to appear as elongated bars separated or in between sister kinetochores that are now clearly separated. During the telophase I/early interkinesis transition, SGO2 and REC8 are not detected at centromeres, while RAD21 persists as elongated bars. In late interkinesis nuclei, RAD21 is still present as elongated bars, and SGO2 reappears as large centromeric signals.

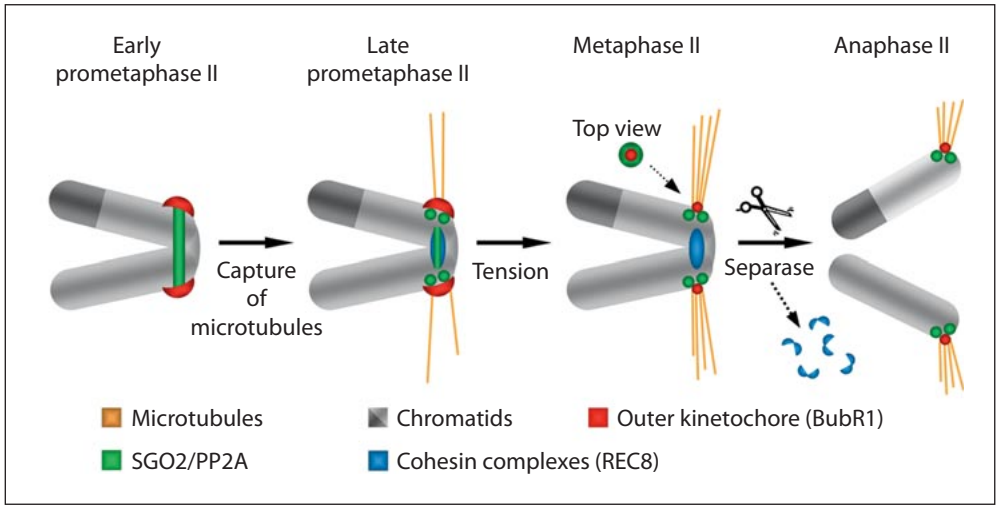

Fig. 6. Schematic representation summarizing current data on the distributions of SGO2/PP2A, the spindle-assembly checkpoint protein BubR1 at the outer kinetochore, and cohesin complexes with REC8 from early prometaphase II up to anaphase II in mouse. The depicted chromosome is telocentric. In early prometaphase II chromosomes, the SGO2/PP2A complex appears at the inner centromeric domain as a band between sister kinetochores which are brightly labeled with BubR1. In late prometaphase II chromosomes, the interaction of microtubules from opposite poles with sister kinetochores generates tension across the centromere, and triggers the redistribution of the SGO2/PP2A complex. At metaphase II, the BubR1 signals at outer kinetochores become faint, and the SGO2/PP2A complex has completely redistributed from the inner centromere domain to appear as two spots (a ring in top view) below each kinetochore. Thus, cohesin complexes containing REC8 become unmasked and able to be cleaved by separase to trigger anaphase II.

vivo mammalian meiosis. *Smc1β* knockout mice present complete male and female infertility, demonstrating the essential role of this cohesin for meiosis [64]. Male meiosis is blocked at pachytene, AE are shortened, homolog synapsis is incomplete, and arm and centromeric cohesion is lost prematurely. Although *Smc1β*$^{-/-}$ oocytes also show problems in SC formation, they progress until metaphase II, but defective sister chromatid cohesion results in massive aneuploidy during meiotic divisions. A recent comparison of oocytes from *Sycp3*$^{-/-}$, *Smc1β*$^{-/-}$ and *Sycp3*$^{-/-}$*Smc1β*$^{-/-}$ double mutants suggested a distinct role for each SMC1 isoform in meiotic AE/LE organization [65].

The characterization of mouse *mei8*, a disrupted allele of *Rec8* induced by chemical mutagenesis, shows that homozygous mutant males and females are sterile, and that REC8 is required to maintain chromosome synapsis, sister chromatid cohesion and chiasma formation during prophase I [66]. Xu et al. [67] deleted all coding exons of the mouse *Rec8* allele by gene targeting. The absence of REC8 function in these mice also provokes infertility in both sexes. These authors found that SC formation in mutant spermatocytes occurs between sister chromatids and not between homolog chromosomes, although SMC3 and RAD21 localization is similar to that of wild-type

in the earlier prophase I stages. This again supports the finding that distinct cohesin complexes function independently throughout the meiotic cycle. In addition to meiotic defects, these mutant mice show a high incidence of embryonic lethality, indicating unknown REC8 functions in somatic cells, in addition to its meiotic cohesion role. This concurs with the human REC8 expression reported in other non-germinal organs [30]. Despite these abnormalities, AE proteins SYCP2 and SYCP3 are present, indicating that AE-like structures are formed in these mutants, although homolog synapsis is not correctly completed.

The absence of AE/LE proteins SYCP3 [68] and SYCP2 [69] induce sexually dimorphic phenotypes; males are sterile due to a disruption in chromosome synapsis and a meiosis block in prophase I, whereas females are subfertile with reduced litter size, with embryo death due to an increase in aneuploid oocytes generated by chromosomal segregation errors. The checkpoints controlling homolog pairing and synapsis are therefore more restrictive in male germ cells [70]. In contrast, the null mutants of TF/CE proteins present distinct behavior relative to sexual dimorphism. Null mutation of *Sycp1* causes sterility in homozygous male and female mice. Most *Sycp1*$^{-/-}$ spermatocytes arrest at pachytene and show problems in repair/recombination and crossover formation [71].

In addition to the initially characterized SYCP1 protein as the major component of CE of SC, three new components have been identified recently: SYCE1, SYCE2 and TEX12. These proteins interact among themselves and with SYCP1 during SC assembly, to form a correct CE [72, 73]. The *Syce2* mutant mouse phenotype is very similar to that of *Sycp1* knockout mice. *Syce2*$^{-/-}$ mice can produce double-strand breaks and initiate recombination processes, but cannot complete SC formation; this results in male and female infertility [74].

These phenotypes suggest inter-dependence among the distinct cohesin, AE and CE axes, such that absence of function of one component of these axes causes SC malformation, almost always provoking meiotic arrest at prophase I. Hamer et al. [75] recently found that meiotic arrest of spermatocytes from *Sycp1*$^{-/-}$, *Sycp3*$^{-/-}$, *Smc1β*$^{-/-}$ mice and from *Sycp3/Sycp1* and *Sycp3/Smc1β* double knockouts (KO) arrest specifically at epithelial stage IV, independently of the cytological end-point observed for each mutant. The authors propose that, while the cytological end-point is determined for chromosomal dynamics within the individual meiotic cells, apoptosis and elimination of anomalous spermatocytes might be induced by an epithelial IV stage-specific paracrine signal. The absence of this male-specific signal could explain the survival of mutant oocytes from KO females beyond pachytene.

Regulators of Cohesin Dynamics: PDS5 and WAPL

Genetic screening, essentially in yeast and fungi, identified cohesin cofactors, proteins that interact with cohesin complexes during the cell cycle. These cofactors are

necessary for cohesin complex dynamics, but are not considered components of the canonical cohesin complex. One of these cofactors is PDS5/BimD/Spo76 [76]. PDS5 interacts with human SA1/STAG1- and SA2/STAG2-containing complexes in somatic cells [77]. In fungi [78, 79] and *C. elegans* [80], PDS5 has an important role in sister chromatid cohesion during mitosis and meiosis. Losada et al. [81] recently characterized two vertebrate PDS5 proteins, PDS5A and PDS5B. PDS5 are large HEAT-repeat proteins that associate with chromatin in a cohesin-dependent manner in both human cells and *Xenopus* egg extracts. They are not required for cohesin association to chromosomes, but are needed for maintaining cohesion. RNAi depletion of PDS5A and/or PDS5B in HeLa cells provokes partial defects in sister chromatid cohesion, and preferentially alters centromeric cohesion in *Xenopus* egg extracts [81]. These chromosomes contain unusually high levels of cohesins, suggesting a role for PDS5 proteins in the regulation of cohesin-mediated cohesion in vertebrate mitosis. Mice lacking PDS5B die shortly after birth and exhibit multiple developmental anomalies that resemble those found in humans with Cornelia de Lange syndrome, indicating a relevant function for PDS5B beyond chromosome segregation [82]. To date, however, there are no known studies of the putative role of PDS5 proteins in sister chromatid cohesion and chromosome dynamics during mammalian meiosis.

Another interesting cohesin cofactor is the product of the previously identified *Drosophila wings apart-like (WAPL)* gene, involved in heterochromatin organization [83]. Two recent reports showed that human WAPL regulates the resolution of sister chromatid cohesion and promotes cohesin complex dissociation by direct interaction with the RAD21 and SA/STAG cohesin subunits [84, 85]. Concurring with its role in chromatid cohesion, WAPL was found on AE/LE in some prophase I stages in mouse spermatocytes [86] and oocytes [87], colocalizing with SYCP3; however, no more exhaustive study has been carried out on the role of WAPL in meiosis. Further cytological and biochemical studies are needed to characterize the role of PDS5 and WAPL during meiosis.

Shugoshins: the Protectors of Centromere Cohesion

In somatic cells from higher organisms, most cohesin complexes from chromosome arms are released from chromatin during prophase and prometaphase by a mechanism that requires phosphorylation of the SA2/STAG2 cohesin subunit [22]. During meiosis, arm cohesion is lost during the metaphase I/anaphase I transition by a process mediated by REC8 subunit cleavage from chromosome arms [88]. One important question is how centromeric cohesin complexes in mitosis are protected from phosphorylation until the metaphase/anaphase transition, and how the centromeric cohesins are protected from separase cleavage until the second meiotic division. This problem was first resolved in fission yeast with the identification of a protein family called shugoshins (Japanese for 'guardian spirit'); Sgo1 and Sgo2 act as

protectors of centromeric cohesion [89]. In fission yeast, Sgo1 is essential in meiosis, whereas Sgo2 has major involvement in mitosis. Shugoshins protect centromeric cohesion in yeast mitosis and meiosis by recruitment of a specific subtype of serine/threonine protein phosphatase 2A, which blocks the cohesin phosphorylation necessary for removal of centromeric cohesion [90, 91].

Human SGO1 and SGO2 also collaborate with PP2A to protect centromeric cohesion in mitosis [for a review see 92]. There are nevertheless few reports in the literature on shugoshin function during mammalian meiosis. The first published study of shugoshin function during mammalian meiosis appeared only a year ago, in which the authors showed the distribution of mammalian SGO2 during male mouse meiosis [62]. SGO2 localization and dynamics in mouse spermatocytes relative to those of RAD21 and REC8 is compatible with a protector function of centromeric cohesion for SGO2 during meiosis I. Interestingly, SGO2 redistributed from a band at the inner centromere domain to a ring below each sister kinetochore during chromosome congression at prometaphase II. This redistribution is sensitive to tension across centromeres, suggesting that SGO2 is a component of the tension-sensing machinery during mammalian meiosis II [62] (figs. 5, 6). Using knockdown experiments, Lee et al. [54] recently showed that SGO2 cooperates with PP2A to protect cohesin REC8 in mouse oocytes and that, in agreement with the previous report, the SGO2/PP2A complex relocalizes in a tension-dependent manner during oocyte metaphase II. It was proposed that SGO2 redistribution would unmask cohesin complexes at the inner centromere domain, which could be then cleaved by separase [62, 54, and reviewed in 93]. This model could explain the specific protection of centromeric cohesion by SGO2 during meiosis I (fig. 5), but the precise molecular mechanisms that trigger SGO2 redistribution remain to be elucidated.

Cohesins and Control of Gene Expression

Although it is not the central topic of this review, we like to briefly summarize accumulating evidence in yeast, flies and mammalian cells indicating that cohesin contributes to chromatin structure, regulation of gene expression, and development [94–96]. Of particular relevance was the identification of hypomorphic mutations in cohesin subunits and cohesin-interacting proteins as the cause of human diseases such as Cornelia de Lange and Roberts/SC phocomelia syndromes [97–100]. The phenotypes of these diseases revealed important, unknown cohesin functions in the control of mammalian gene expression. Unlike yeast, most cohesin-binding sites in human cells are associated with the zinc-finger protein CCCTC-binding factor (CTCF), which is required for transcriptional insulation [101–103]. Although further experiments are needed to establish the molecular mechanism by which cohesin contributes to transcriptional insulation, these results suggest that cohesin complexes are responsible for the formation of a specific chromatin structure that causes insulator effects.

In addition, two groups using distinct approaches demonstrated that cohesin regulates the correct morphogenesis of *Drosophila* neurons [104, 105]. In this case, cohesin facilitates transcription of the *ecdysone receptor (Ec-R)* gene [104]. This is the opposite of the effect previously described as insulation factor, but similar to that described for STAG2 as a transcriptional co-activator in human cells [96]; this indicates that the role of cohesin in transcription regulation might be gene-dependent. These studies have been central to progress in the insight of the participation of chromosomal structural components, such as cohesins, in gene expression.

Concluding Remarks

A decade after their identification, the cohesins are considered key players in the maintenance of genome integrity during cell division. During meiosis, distinct cohesin complexes formed by the combination of different meiosis-specific and non-specific subunits regulate chromosome dynamics, and are essential for the correct progress of germ cells through prophase I. Based on cytological and biochemical studies, STAG3 has been proposed to be an arm cohesion-specific molecule, whereas REC8, RAD21 and SMC1β contribute to arm and centromeric regions, but do not colocalize exactly at the same chromatin sites. The recent discovery and characterization of the shugoshin proteins, and their collaboration with phosphatase PP2A in centromeric cohesion protection during mitosis and meiosis from yeast to mammals, revealed high conservation of chromosome cohesion regulation throughout evolution. Generation and characterization of knockout and knockdown models in recent years have been crucial in clarifying the in vivo role of cohesins.

How many cohesin complexes do participate in maintaining arm and centromere chromosome cohesion during meiosis? Do they have distinct specificity in their chromatin binding sites? Is the loading of different cohesin complexes regulated temporally throughout the meiotic cell cycle in mammals? What are the functions of PDS5 and WAPL cohesin cofactors in mammalian meiosis? Are both SA1/STAG1- and SA2/STAG2-containing complexes involved in gene regulation function, or do they have distinct specificities depending on the gene? Do cohesins have similar functions in germ cells, in some way controlling expression of certain genes during meiosis? These are some of the exciting questions that remain to be addressed.

Acknowledgements

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Variation in Patterns of Human Meiotic Recombination

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Abstract

In the last 30 years it has become evident that patterns of meiotic recombination can be highly variable among individuals. The evidence comes from both low and high resolution analyses of hotspots of recombination in human and other species. In addition, a comparison of the recombination profiles in closely related species such as human and chimpanzee reveals essentially no correlation in the position of hotspots. Although the variation in hotspots of meiotic recombination is clearly documented, the mechanisms responsible for such variation are far from being understood. Here we will review the available evidence of natural variation in meiotic recombination and will discuss potential implications of this variation on the functional mechanisms of crossover formation and control.

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Meiosis is a key process in sexual reproduction and meiotic recombination is an intrinsic part of meiosis in most organisms. In mammals recombination intermediates provide a structural basis for the accurate segregation of chromosomes and meiotic recombination is necessary for completion of meiosis. A serious consequence of defects in meiotic recombination is aneuploidy. An important evolutionary consequence of meiotic recombination is the generation of genetic diversity.

A widely established paradigm of the initiation of meiotic recombination is the double-strand break (DSB) initiation model [1]. This model postulates that programmed DSBs introduced in the leptotene stage of the first meiotic division are used to initiate genetic recombination in all organisms including mammals [1–3]. These DSBs are then processed by a variety of meiosis-specific and non-specific proteins and result in the formation of crossover and non-crossover products. Although the molecular basis of crossover formation is being increasingly unraveled, the mechanisms of the regulation of meiotic recombination remain largely unknown. It is clear, however, that meiotic recombination is a highly non-random process. In most organisms that have been carefully examined meiotic DSBs are tightly clustered in hotspots

instead of being uniformly distributed [4–7]. The hotspots of recombination themselves are also distributed non-randomly in the genome. The difference between the recombination rates inside hotspots and in surrounding 'cold' regions exceeds several thousand-fold. This preferential localization of meiotic crossovers to small regions of the genome strongly indicates that the site selection of programmed DSBs in meiosis is tightly regulated. Many genomic features are correlated with hotspots, but the correlations are weak and generally become weaker at higher resolution [4, 6–9]. Many, but not all, of the observed correlations are associated with a higher GC content. Thus, our ability to predict hotspots is still poor and the rules defining where hotspots are located in the genome remain mysterious.

Studies of meiotic recombination in humans are difficult. The large size of the genome and the relatively low frequency of human meiotic recombination (~1 cM/Mb) compared to budding yeast (~300 cM/Mb), for example, makes direct studies of recombination intermediates impossible using conventional molecular biology methods, such as gel electrophoresis. Thus the experimental techniques that are suitable for the analysis of meiotic recombination in mammals target identification and quantification of final recombination products, recombinant chromosomes. Although it is possible to detect all meiotic crossovers on a genome-wide scale, such studies have been limited to lower than 1 Mb resolution. Presently, the measurement of crossover frequency at high resolution is only possible on a limited scale. Thus, there is a potential problem between the small size of the hotspots that are believed to be the minimal functional unit of recombination and the inability to perform comprehensive analyses at high resolution. This makes a genome-wide analysis of mammalian meiotic hotspots rather challenging.

Accumulating evidence indicates that there are differences in meiotic recombination profiles between individuals and populations [see 10–12 for reviews]. Such differences are observed both at low resolution and at the level of individual hotspots. In this review we use 'low resolution' as the designation for the analyses that cannot resolve individual hotspots and 'high resolution' as the designation for the techniques that can separate individual hotspots or at least approach such resolution. For the human genome the boundary between high and low resolutions corresponds to around 100–200 kb. Here we will review and summarize the available evidence for the variation in hotspots of meiotic recombination in mammals with an emphasis on high resolution data. Male meiosis is very different from female meiosis. For example, the total number of crossovers in female meiosis is more than 50% higher than in male meiosis and while female crossovers are more frequent in centromeric regions of chromosomes, male crossovers are more common near telomeres [10]. These differences suggest a substantial divergence in the global regulation of meiotic recombination between sexes. Thus, we find it inappropriate to consider sex-specific differences in patterns of meiotic recombination as polymorphisms and do not discuss such sex variation in this review. Interested readers are referred to the excellent review by Audrey Lynn et al. [10].

Evidence of Variation at Low Resolution

There are two major approaches used to analyze meiotic recombination at low resolution: direct visualization of recombination intermediates and analysis of genetic linkage maps. The inter-individual variation in meiotic recombination frequency in humans was first noticed by direct observation of chiasma (see [13] for overview and analysis of a number of early works). Later studies confirmed and advanced earlier findings [14]. The development of immunochemical techniques for visualization of proteins associated with crossovers significantly facilitated the analysis [see 10 for review]. In these assays for the detection of crossovers antibodies against the MLH1 protein are used most frequently, but not exclusively. These studies clearly prove that the total number of recombination foci varies significantly among individuals both in male [15–17] and female [18, 19] meiosis [for a detailed review see 10]. The observed numbers have been reported to be from 43 to 56 crossovers per cell and 50 to 95 crossovers per cell for male and female meioses, respectively. In addition, at least a three-fold variation in the numbers of crossovers [16, 19] was reported for cells isolated from the same individual.

An alternative to direct observation of crossovers using cytological methods is the comparison of genomic sequences of chromosomes that completed meiosis with those of parental chromosomes. Determination of genotypes at polymorphic markers allows mapping crossover position with a resolution that depends mostly on marker density. The analysis of the recombination frequency between polymorphic markers captured as a genetic distance in linkage maps provides a sex-specific, population-averaged measure of meiotic recombination. Similarly to cytological methods, genetic maps constructed using short tandem repeats (STR)-based genotyping approaches show that recombination rate varies among individuals [20–23].

In addition to the inter-individual variation in recombination frequency or total map length, linkage maps provide some evidence of recombination rate variation in specific regions of the genome [24, 25; for a review see 10], although some of these findings have not been confirmed [26]. We must say however that classic linkage maps may not be very well suited for the regional comparison of recombination rates between populations. Potential problems include the effects of errors in genotype definition, suppression of recombination due to the presence of polymorphic inversions and the incorrect ordering of markers [see 24 for discussion]. A recent application of classic linkage analysis combined with application of a novel statistical approach proved the existence of extensive variation in meiotic recombination [27]. Cheung and coauthors performed a comprehensive analysis of 38 CEPH (Centre d'Etude du Polymorphisme Humain or Center for the Study of Human Polymorphisms, an international genetic research center where a collection of immortalized cell cultures from large reference families has been created) families and identified 17,461 genetic crossovers at roughly 0.5 MB resolution in 34 mothers and 33 fathers [27]. They found a highly significant variation of recombination rate among individuals in both

males (range 16.9–28.9 recombination events per meiosis) and females (range 27.5–46.4 recombination events per meiosis) and strong evidence of positional variation in individual recombination rate profiles at 5 Mb resolution [27]. Similar findings were reported for high-resolution pedigree-based linkage analysis of a part of chromosome 22 [28].

An extension of linkage analysis particularly well suited for studies of recombination is called single sperm genotyping. In this case, genotypes are determined not in multi-generation nuclear families, but rather from multiple sperm cells from the same individual. A comparison of obtained genotypes allows determining the proportion of recombinant molecules and thus the frequency of recombination at a given locus. There are two major modifications of the method. In one, single sperm cells are first separated and then genotypes are determined for the region of interest for each of the individual cells [29]. In the other, potentially more sensitive approach, the fraction of recombinant molecules is calculated in small pools of sperm cells (for reviews on sperm genotyping see [4, 29, 30]). Although lately sperm genotyping is mostly used for high resolution analysis, the modifications of sperm genotyping where individual cells are analyzed separately can be efficiently applied to analyze regions of arbitrary size. Single-sperm genotyping applied to megabase-scaled loci found significant variation in position-specific recombination rates among individuals [10, 31, 32].

Thus, we can see that even at the relatively low resolution provided by cytogenetic and genetic mapping, variation in meiotic recombination can be clearly detected. One however should be cautious in interpreting these results. The analysis at low resolution provides an average of recombinational activity of tens to perhaps hundreds of individual hotspots. In addition, the use of classic linkage maps is associated with the potential artifacts mentioned above.

Evidence of Variation at High Resolution – Computational Approaches

A significant weakness of the analyses performed at low resolution is the inability to study recombination on a level of the individual functional units, meiotic hotspots. In contrast, high resolution analysis has the power to assess the functional activity of individual hotspots. Two approaches are frequently used to define profiles of recombination at high resolution. The first approach is the reconstruction of high resolution recombination rate profiles from genetic variation data. The second approach, mentioned above, is sperm genotyping.

The first approach uses the genetic variation found in populations to estimate recombination rates. What is now seen as single nucleotide polymorphisms (SNP, the most frequent kind of sequence polymorphism) was at some time in the past a rare mutation in an ancestral chromosomal context. These ancient chromosomes have been mixed in thousands of generations to produce present-day chromosomes, but

the historic association of SNPs is still detectable. These patterns of non-random association of genetic markers, called linkage disequilibrium (LD), are shaped by past recombination events [33]. Computer modeling allows reconstructing the population history backwards in time and estimating the likelihood of a given recombination frequency between pairs of polymorphic markers. An ever increasing number of methods have been developed for the estimation of recombination rates from population variability data, the most accurate of which are based on coalescent reconstruction [for review see 34, 35]. One potential drawback with many of these methods, in particular those that are more accurate in estimating rates, is the extreme computational demands. Full coalescent reconstruction is absolutely prohibitive from a computational point of view. Several approximations to the calculation of full-likelihood have been introduced (two programs, LDHat [36] and Phase [37–39], are most popular, and see [34, 35] for discussion). Although these approximate methods are still computationally-intensive, rapidly increasing performance of modern computers makes it possible to perform such calculations even on a genome-wide scale and such methods have been applied successfully to calculate high resolution recombination rate maps of the human genome [8, 36]. The advantages of computational methods are speed, throughput and cost-effectiveness. It is relatively easy to collect DNA samples from 30–50 individuals and determine their genotypes using modern genotyping techniques. These genotype data can then be used to calculate recombination rate profiles.

The variation between population-specific recombination rate profiles has been documented in several studies [8, 40–45]. The variation in calculated profiles of recombination was first comprehensively studied by Clark and coauthors [40]. They found evidence of population heterogeneity at ~100-kb resolution in many of the 538 SNP clusters studied [32]. Later studies were performed at higher resolution allowing analysis of individual hotspots. For example, by performing a computational analysis of 74 genes resequenced in 47 individuals as a part of the SeattleSNP program (http://pga.gs.washington.edu/), Crawford et al. found evidence of hotspots in 35 genes and in 16 of these 35 genes (45%) a hotspot was found only in one population [42]. A more sensitive recent analysis of a slightly larger set of SeattleSNP genes again found that 35% (43 out of 121) of all hotspots were detected only in one population out of two [43]. What has been missing from studies performed so far is an accurate unbiased estimate of the statistical significance of the observed differences on a genome-wide scale.

An interesting application of the LD-based computational methods is the comparison of profiles of recombination in closely related species [46–49]. In these studies the authors have reconstructed recombination rate profiles in the orthologous regions of the genome in human and chimpanzee based on population surveys of genetic variation. Despite a 98.7% identity in DNA sequence, there is no correlation in the positions of hotspots in up to 14 Mb of sequence [46–48]. Thus, hotspots of meiotic recombination have completely changed their positions in the ~7 MYR since

the split between the human and chimpanzee lineages. At the same time, in an apparent contradiction to previous findings a recent study has shown that hotspots are found in the same location in paralogous genomic loci with an age of duplication preceding the human-chimpanzee split [50]. It is unclear, however, how general is such a conservation in hotspot position.

An intrinsic problem associated with the computational methods discussed is the fact that they are based on calculating population-averaged recombination rates from a sample of individuals. Thus, measuring the individual-specific recombination activity is impossible in principle. Another caveat is the fact that the inaccuracy in defining recombination rates from sequence variation data using coalescence-based approaches is rather high [8, 36, 51]. Thus, it is likely that many or even the majority of the observed differences between population-specific recombination rates do not reflect true biological variation. In addition, the definition of confidence intervals of recombination rate estimates with respect to true biological rates is not trivial [36, 51]. Another potential problem is that the majority of coalescent-based methods are based on a very simple model of DNA recombination that assumes that hotspots are completely conserved in a population. We now believe that this is an oversimplification and there is a great deal of variation in hotspot strengths among individuals. Recently introduced methods try to address this issue of the possible variability in hotspots and incorporate the heterogeneity explicitly in the likelihood calculations [52, 53], however, this is still an area of active development. The development of more realistic recombination rate models that incorporate hotspots explicitly, as such [54] will also improve the quality of predictions and the power of analysis.

Evidence of Variation at High Resolution – Experimental Approaches

A dominant experimental technique used to analyze meiotic recombination products at high-resolution is single-sperm genotyping [for review see 4, 29, 30]. It allows the direct measurement of recombination frequency at a given location by calculating the proportion of recombinant molecules in a sample of DNA purified from spermatozoa or oocytes. There are two major variants of the method. Proposed by the group of Norman Arnheim in late 1980s [55, 56] the first variant of the method is based on the determination of the genotypes of individual cells. In one of the most comprehensive efforts in single sperm genotyping, 20,031 cells were genotyped across the 3.3 Mb major histocompatibility complex (MHC) region on chromosome 6 and the authors found strong evidence of variation in recombination rates among 12 donors at a resolution approaching single hotspots [21].

An intrinsic problem associated with the testing of individual cells one by one is the lack of sensitivity in measuring recombination rates. The number of the cells that are assayed roughly determines the frequency of crossovers that can be detected.

Typical hotspots recombine in less than 1 cell per thousand [4]. Thus, to somewhat reliably measure the activity of these hotspots at least several thousand cells must be analyzed. This intrinsic problem led to the development of pooled DNA sperm genotyping [57] which became the ultimate standard in high-resolution studies of meiotic recombination in mammals [for review see 4, 29, 30]. This method is based on the application of allele-specific PCR to determine the proportion of recombinant molecules out of all sperm DNA molecules. This modification achieves a superior sensitivity where even the weakest hotspots can be assayed but the drawback is the smaller region that can be analyzed in a single assay. The limit is imposed by the size of the PCR product.

An exceptionally high degree of polymorphism has been observed in male-specific profiles of recombination at the level of individual hotspots by pooled-sperm genotyping [58–63; and for review see 11]. 6 out of 16 hotspots identified by high resolution sperm genotyping in the MHC2 region on chromosome 6 and in the MS32 locus on chromosome 1 by the group of Alec Jeffreys were found to be polymorphic [58–62]. In addition, a recent analysis of a 103-kb region on chromosome 21 identified 3 hotspots and 2 of them were polymorphic [63]. In total, the recombinational activity of 8 of 19 hotspots of recombination studied in detail is polymorphic. Thus, polymorphic activity of meiotic hotspots in human appears to be the rule rather than the exception.

Some interesting facts are emerging from a comparison of the computational reconstruction of recombination profiles and experimental data. Although, overall coalescent reconstruction is rather accurate and correlates well with experiments, there are exceptions. For example, using sperm genotyping hotspots have been detected in regions where no hotspots were predicted computationally [59]. The opposite situation has also been seen: A hotspot predicted by computational methods has not been detected in some men [64]. These observations can be explained by polymorphic variation in hotspots or their rapid evolution.

The main disadvantage of sperm genotyping is the relative complexity of the method and the inability to analyze larger genomic regions. At present, the longest region that has been studied at a time is 103 kb [63]. That leads to the most significant drawback of high-resolution studies, which is genome coverage. While several comprehensive genome-wide studies have been performed to date at low resolution, high resolution analyses (sub 100 kb) cover less than 1% of the genome. Studies performed at the level of individual hotspots cover less than 1 Mb in total. Considering the high regional variation in rates of recombination one should be careful in trying to generalize the limited data available to date. Thus, the extent of genome-wide variation of high-resolution recombination profiles is still not known. However, we still think that all the evidence suggests that the inter-individual differences in profiles of recombination are high. A potential for further development of sperm genotyping techniques lies in the application of high performance next-generation sequencing and genotyping technologies to single cell analysis.

Implications on the Mechanisms of Recombination

What kind of insights on recombination mechanisms can we gain from studies of the variability of recombination rate profiles? First of all, a model for crossover formation in humans must be able to explain the high degree of inter-individual variation in recombination profiles. There is a high degree of polymorphism in hotspots of recombination detected both by computational and experimental methods. The comparison of position of hotspots in human and chimpanzee also suggests that they evolve at a much higher rate than DNA sequence. In addition, the model must accommodate that while fine-scale recombination rates are highly variable, they are relatively conserved at low resolution [26, 64].

Although the influence of short DNA motifs on hotspots of recombination is unquestionable [6–9], it would be difficult to reconcile this degree of variation if the recombination is determined solely by the local DNA sequence. The more likely explanation is the involvement of epigenetic factors, nuclear architecture and/or distant DNA elements in the control of recombination [46, 47]. In such case one can easily imagine two layers of regulation where first the recombination is regulated at the level of chromosome domains and then at a finer scale where individual hotspots are chosen.

Independent evidence for a non-local mechanism determining recombination activity comes from an examination of the 'recombination hotspot paradox' [65] which consists in the contradiction between the relative persistence of hotspots and a strong pressure on intense hotspots to self-destruct due to the deletion of the allele that initiates DSB formation at the hotspot. A detailed population-genetic analysis does not provide a satisfactory explanation for the persistence of hotspots if they are determined solely by the local DNA sequence [52, 53, 66]. The presence of strong hotspots in humans is proven experimentally, however. This contradiction again suggests that the strength of a hotspot is determined by non-local elements that would not be affected by gene conversion.

In yeast the importance of chromatin structure in determining the positions of meiotic DSBs is clearly established. A number of detailed studies of the regulation of meiotic DSB formation in yeast provide compelling evidence for the complex interplay and essential involvement of chromatin structure in hotspot formation [7, 67–70]. A recent study, for example, demonstrated that the loss of histone deacetylase dramatically changes the distribution of meiotic DSBs [71].

Strong evidence for the epigenetic control of recombination in human comes from two recent articles where male recombination profiles were studied using sperm genotyping [58, 63]. In the first work Neumann and Jeffreys found that the activity of hotspots is not determined by local sequence. Some men sharing the same haplotype have an active hotspot while it is suppressed in others [58]. In the study by the group of Arnheim and colleagues, the authors found that the decrease of the activity in one hotspot, as seen in yeast [68, 69], is compensated by an increase in one nearby [63]. This observation supports the idea that there is a regional control of recombination.

An essential involvement of non-local elements in control of meiotic recombination was also seen in mouse [72]. Thus, we see that there is considerable evidence in support of the epigenetic control of meiotic recombination in mammals.

Conclusions

In summary, the combined evidence shows that there is a high degree of inter-individual variation in hotspots of meiotic recombination in humans, but the mechanisms responsible for this variation are mostly unknown. Further studies and comprehensive high-resolution analysis on a genome-wide scale in particular will allow us to more accurately quantify the variation in hotspots of meiotic recombination among individuals. However, it seems likely recombination may be one of the most variable biological processes. It would be very interesting to untangle the regulatory mechanisms responsible for such variation from the conservation of low-resolution recombination profiles and explain how this highly variable process can operate under the multiple constrains characteristic to meiosis.

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Maternal Origin of the Human Aneuploidies. Are Homolog Synapsis and Recombination to Blame? Notes (Learned) from the Underbelly

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Abstract

Aneuploidy is the leading cause of mental deficiency in human newborns. Indirect studies suggest that, in most of the cases, the extra chromosome comes from an inaccurate meiotic division. But, particularly, all results seem to indicate that oogenesis is more prone to err than is spermatogenesis. Unfortunately, due to the time-frame in which meiosis takes place in the mammalian males and females, most of the studies performed so far have focused on analyzing male meiosis. Recently, some studies focusing on human meiosis have been published. Some of them revealed important sex-specific differences that may be involved in the predominant involvement of the human female in the genesis of aneuploidy. In this article, the current knowledge we have about human female meiotic synapsis and recombination is summarized and we try to relate it to the human aneuploidy origin. Copyright 2009 © S. Karger AG, Basel

In humans, it is estimated that there is a high percentage (10–25%) of aneuploid conceptions. These aneuploidies are mainly autosomopathies caused by errors produced during the first stages of oogenesis [1]. Female meiosis (much more than male meiosis) is error-prone and we also know that this flaw increases exponentially with age. The origin of the three most frequent, human life-compatible autosomopathies (trisomy 13, 18 and 21) involves errors in the egg, in more than 85% of the cases. Genotyping studies of polymorphic DNA markers have shown that most of them arise through chromosome mis-segregation in meiosis I, as is the case of trisomy 13 and 21. In contrast, trisomy 18 is mainly (55%) due to non-disjunction in meiosis II [2–13].

Although the origin of human aneuploidy is predominantly maternal most meiotic studies have been performed in spermatocytes. However, few groups have attempted to study human female meiosis in order to elucidate whether there are

Table 1. Pre-meiotic non-disjunction and pairing-error rate in human oocytes

References	3 univalents at leptotene				2 univalents at pachytene			
	Chr. 13 (%)	Chr. 18 (%)	Chr. 21 (%)	Chr. X (%)	Chr. 13 (%)	Chr. 18 (%)	Chr. 21 (%)	Chr. X (%)
[20, 22, 51]	–	0.0	0.0	0.1	–	0.0	0.4	0.0
[23, 24]	0.0	0.6	0.2	0.0	0.1	0.2	0.3	0.0

some meiotic differences between sexes that could explain such a huge gap in the aneuploidy rates between oocytes and spermatocytes.

Many differences concerning mainly homolog chromosome pairing and synapsis and the recombination process between male and female meiosis have been described suggesting that these phenomena may be involved in the differences observed in the origin of human aneuploidy. Differences in pairing-synapsis include features such as oocyte synapsis initiation from both subtelomeric and interstitial sites versus synapsis initiation exclusively at terminal sites in spermatocytes; higher incidence of pachytene oocytes showing structural chromosome aberrations and chromosome interlockings; delayed female bouquet evolution compared to the male [14–17], etc… However, in agreement with Hunt [18], all of these pairing-synapsing alterations, sometimes only variations, could explain some atresia phenomena but, in any case, they would not have enough importance to provoke real and big problems in pushing chromosomes together and/or connecting them. In this matter, Tease et al. [19] went further by posing that synapsis difficulties might not be the initiation event for atresia either. In fact, when studying the meiotic progression in oocytes with a paracentric inversion, Cheng et al. [20] observed this to be normal.

Analysis of pairing-synapsis of chromosomes 13, 18, 21 and X, which are involved in some of the most common trisomies in human newborns and in the aneuploidies shown by oocytes at metaphase II, has shown that:

(a) the occurrence of pre-meiotic non-disjunction events has been described in human oocytes [21–24]. Although being very rare, its rate is different for each chromosome studied. For example, chromosomes 18 and 21 exhibit pre-meiotic non-disjunction, while chromosome X does not (table 1).

(b) there is an interchromosomal interfering effect described for chromosomes 21/X and 21/13, meaning that the presence of an extra chromosome 21 somehow disturbs chromosomes X and 13 synapsis. However, this phenomenon seems to be chromosome specific, as it has not been noted for chromosomes 18/13 [14, 21, 23].

(c) the pairing-error rate at pachytene stage seems to be equivalent (0.14%) to all analyzed chromosomes (13, 18, 21 and X) independently of chromosomal size, morphology or DNA content [21–24] (table 1).

Table 2. Chiasma counts in human spermatocytes

References	Samples	Spermatocytes analyzed	Chiasmata numbers	
			Range	Mean ± SD
[36]	11	2,639	33–66	50.6
[34]	21	516	43–62	52.7
[35]	1	41	43–60	50.6 ± 3.9
[33]	7	408	50–54	51.3 ± 1.4
[32]	6	91	32–58	45.3 ± 4.5

Therefore it can be concluded that despite the occurrence of all these disturbing facts, the pairing-error rate is very low in human oocytes and therefore it seems to be a very efficient process that ensures the encounter of the homologs.

At this point, how can we explain the contradiction between this very low error rate (0.14%) and the high rate (from 15–25%, depending on the studies [26–28]) of unbalanced oocytes II found in humans? Does this mean that the implication of the pairing-synapsing processes themselves in the origin of the high number of unbalanced oocytes II is minimal, as has been defended by Robles et al. and Roig et al. [23, 29]? We have to conclude that the differences in the rate of aneuploid oocytes compared to spermatocytes cannot respond to errors in the pairing-synapsis process.

When looking for answers to the question: 'Why do we conceive so many aneuploid offspring?', the investigations at this moment are moving to other landmarks of meiosis: recombination and segregation. In this review we summarize the current knowledge we have of the recombination process in human meiocytes and try to understand whether this can be involved in the origin of human aneuploidy. Unfortunately, due to limited space, the effect of chromosome segregation will not be mentioned here, as we believe it deserves a complete revision.

Meiotic recombination is the procedure that promotes genetic diversity from generation to generation. In addition, it plays another essential function: it tethers homologous chromosomes together in order to achieve a proper segregation at metaphase I. In this sense, it has long been proposed [30] that chiasmata, the cytological evidence of recombination, play a central role in the genesis of aneuploidies. In fact, in model organisms, it has been recognized that when having problems in the recombination process, there is an increase of non-disjunction events. However, due to the difficulties to obtain human samples but, mainly, because of the demanding methods to find evidence of crossingover, only few investigations aiming to study the exchange between homologous chromosomes have been performed in human meiocytes [9, 11, 31–40]. Human male recombination has been analyzed and, at this moment, at least a few of the main questions on this topic have been answered (tables 2 and 3).

Data from direct analysis of human spermatocytes by chiasmata (at diplotene-metaphase I) or by MLH1 foci (at pachytene) count are very similar to each other and, at

Table 3. MLH1 foci counts in human meiocytes

References	Samples	Meiocytes analyzed		MLH1 foci at pachytene	
		male	female	Range[a]	Mean ± SD
[31]	1	46	–	41–59	50.9 ± 4.4
[9]	14	1,384	–	34–66	49.1 ± 4.8
[11]	25	2,182	–	34–66	49.8 ± 4.8
[37]	1	100	–	38–62	49.8 ± 4.3
[38]	5	224	–	36–63	48.8 ± 2.3
[39]	2	213	–	32–63	48.5 ± 3.6
[40]	10	1,000	–	n. m.	49.7 ± 5.0
[42]	1		3	88–104	95.0 ± 12.3
	1	–	6	62–94	77.3 ± 13.0
	1		5	53–87	71.6 ± 12.5
[43]	1	–	95	48–102	70.3 ± 10.5
[44]	9	–	131	n. m.	50.3 ± 24.7

[a]n. m.: Not mentioned.

the same time, they fit the expectations predicted by Broman et al. [41] from indirect studies in the only systematic linkage-analysis of genome-wide levels of recombination published to-date. The obtainment of the coefficient of variation from the means and the standard deviations demonstrate some kind of variability both within and among individuals (3–10%) in both analyses (MLH1 foci and chiasmata). Nevertheless, it is lower than the one described by indirect studies (9–12%). From this information, the existence of some heterogeneity both between and among individuals is inferred.

The message that comes from human female recombination studies (direct and indirect approaches) is much more difficult to summarize. First of all, it is important to mention that there are no published oocyte chiasma counts due to the extreme complexity of the analysis itself. Second, up to now, there are only very few human female MLH1 analyses. And third, looking at these few data, it is easy to see that MLH1 foci counts do not harmonize among themselves (table 3). In addition, only the results from two studies [42, 43] fit with the expectations predicted by indirect studies, while mean numbers from two different laboratories go down to half of the estimations predicted. Anyway, in spite of the contradictions shown above, there is clear agreement among authors regarding the striking heterogeneity within and among female human samples. In this sense, it is important to be aware that considering global heterogeneity found by Lenzi et al. [44], and P. Robles and M. Garcia Caldés (unpublished data), there are a lot of oocytes, 18–30%, with chiasma counts

Table 4. MLH1 focus count variation among different chromosomes (data calculated from [43])

Chromosome	% of MLH1 foci per bivalent at pachytene					
	0	1	2	3	4	5
21	3.5	69.8	26.7	–	–	–
18	1.5	6.0	53.2	35.8	4.5	–
13	–	3.1	48.4	43.8	4.7	–
X	–	–	26.1	39.1	21.8	13.0

below the minimum value to assure proper attachment of homologs until metaphase I (i.e., 2 chiasmata per metacentric and submetacentric chromosomes and 1 chiasma per acrocentric chromosome). These oocytes below the threshold could be one of the main reasons for such a high quantity of unbalanced metaphase I oocytes. Nevertheless, we cannot exclude the possibility that, for any technical reason, the number of MLH1 foci we observe in human spread oocytes, at any particular point in time, is not the total number of foci the cell is going to present. Thus, in human oocytes the MLH1 counts could underestimate the number of COs. Unfortunately, so far we do not have any way to assess either of these two hypotheses.

It has to be taken into account that the global heterogeneity in terms of MLH1 counts seen in human oocytes is not uniformly distributed among all the chromosome pairs [43]. Only the largest meiotic chromosomes show high variability. The variability depends on the morphology of the chromosome and, maybe more importantly, on the length of the SC (table 4). For example, the longer the chromosome, the more likely it is to present different numbers of MLH1 foci in different cells (table 4).

Researchers working in human meiosis have made particular efforts in characterizing the recombination process on chromosome 21 with the aim of understanding the implication of meiotic recombination in the origin of the most prevalent autosomopathy in newborns, Down Syndrome. In summing up all of the published results about human recombination on chromosome 21 [2, 11, 23, 37, 40, 43, 45, 46] and considering their Coefficients of Variation, their recombination point numbers and their distribution obtained by human direct and indirect approaches, it can be concluded that:

(a) There is a slight heterogeneity in the male as well as in the female (table 4). Hence, the contribution of chromosome 21 to the high global MLH1 heterogeneity in human oocytes is minimal. In fact, chromosome 21 shows the lowest heterogeneity among all chromosomes studied so far. Indeed, Brown et al. [47] have suggested that a different threshold exists for each chromosome that ensures correct disjunction at metaphase I. In this sense, the minimum chromosome 21 threshold is almost always accomplished.

(b) There is non-random distribution of recombination points, either male or female, but, at the same time, there is an evident sex-difference in its placement:

Table 5. MLH1 focus counts on chromosome 21 in human meiocytes at pachytene

References	Samples	Meiocytes		% Bivalents			Frequency
		male	female	zero	one	two	
[45]	–	131[a]	–	0.0	90.9	9.1	1.09
[2]	–	262[b]	–	0.0	97.0	3.0	1.03
[11]	25	119[c]	–	0.0	98.3	1.7	1.02
[37]	1	50[c]	–	0.0	100.0	0.0	1.00
[39]	2	213[c]	–	4.7	93.9	1.4	0.97
[40]	–	262[c]	–	2.1	–	–	–
[43]	1	–	86	3.5	69.8	26.7	1.23

[a]Homolog chromosome 21 in spermatocytes at the diplotene/metaphase I stage.
[b]Paternally inherited chromosome 21.
[c]Homolog chromosome 21 in spermatocytes at the pachytene stage.

terminally (in spermatocytes) *versus* interstitially (in oocytes) [2, 43, 45]. It has been defended in mice that there is a close relationship between sex-specific synaptic patterns and recombination distribution. In addition, sex and not genotype seems to be a primary determinant of meiotic recombination-distribution patterns in mammals [48]. In any case, nowadays, there are not enough human data to either corroborate or reject these statements.

(c) Bivalents 21 without an MLH1 focus are described both in males and females. But more interestingly, the proportion found in females is comparable to that reported in males (table 5).

(d) There is an increase of CO frequencies in females in relation to the ones observed in males (table 5). This enlargement could be due to the increased SC length of bivalent 21 in the females that would allow the presence of a higher amount of bivalents displaying two MLH1 foci compared to the male.

It is commonly accepted that stability of homolog-crossover configuration depends on the placement and number of chiasmata (reviewed by Lamb and Hassold [49]). Bivalents either without chiasmata or with chiasmata located at or near the telomere are less efficient in holding homologs together until metaphase I, thus increasing the risk of meiotic non-disjunction.

For all of these reasons, it can be assumed that chiasma configuration over chromosome 21 is more stable in human oocytes than in human spermatocytes. Thus, the higher maternal trisomy 21 origin cannot respond to:

(a) A high chromosome 21 pairing-error rate, as we have understood from pairing-synapsing data.

(b) Unstable chiasma configurations by themselves. Because, as has just been mentioned above, chiasma configurations on female bivalent 21 are usually stable.

(c) Lower chiasma counts. In any case, the human female does not have more achiasmatic bivalents than does the human male.

Therefore, it can be concluded that, at least for chromosome 21, both oocytes and spermatocytes are equally efficient in pushing together, connecting and exchanging homologous chromatids. However, data, mainly from knockout mice, indicate dissimilar cell-checkpoint behavior [13]: while, in general, meiotic checkpoints in mouse spermatocytes are very stringent, probably due to the need to form a functional sex body, mouse oocytes are able to progress through meiosis even when bearing meiotic alterations, leading to the conclusion that meiotic checkpoints are less effective in the female oocyte. As Tease et al. [19] defended, it remains to be determined whether a comparable tolerance to synaptic error in combination (or not) with failure of recombination is also present in human oocytes.

When looking at the origin of human aneuploidy we have also to take into consideration the effect of environmental factors on meiocytes and the way that oocytes and spermatocytes face them. In this sense, the only important known factor empirically demonstrated is age. The effect of age is much more devastating for oocytes than for spermatocytes, where advanced maternal age or, to be more exact, physiological ovarian age, has been linked to increased rates of oocyte aneuploidy.

Indirect studies by Lamb et al. [50] have revealed that chromosome 21 exchanges in oocytes of young mothers of Down Syndrome children were unstable crossovers and were predisposed to non-disjunction, while in older mothers a wide range of exchange configurations was found leading to the conclusion that unstable chiasmata (in agreement with Lamb et al. [2], 1st hit) are predisposed to mis-segregate, the stable configurations would need adverse conditions (maternal advanced age, 2nd hit) for non-disjunction. And, of course, in both cases the action of an inaccurate cell-checkpoint is needed in order to let the oocyte pass to later gamete stages. In any case, it is important to notice that the exact relationship between aneuploidy and advanced maternal age varies among trisomies and currently, we cannot generalize this hypothesis to the rest of the chromosomes.

Hence, in trying to find the reasons for such high meiotic errors that oocytes show, the main difference that we have been able to find between both gametogenic processes is not in the prophase meiotic stages, but rather in the regulation procedure guidelines that meiocytes employ to check the development of the process faced with environmental factors and, in this sense, to control the final gamete.

In any manner, the current knowledge on human female meiosis represents the first step of the long journey in human meiosis research.

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Inverted Meiosis: The True Bugs as a Model to Study

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Abstract

Most of the meiotic literature is based on species with monocentric chromosomes, however meiosis in protoctist, plant and animal species with holocentric chromosomes is less characterized. In some cases, an inverted meiotic sequence is claimed to occur, in which segregation of homologs is postponed until the second meiotic division. Additionally, other features also deserve interest, namely: (i) the different behavior of sex chromosomes if compared to that of the autosomes; (ii) the absence of a canonical kinetochore structure; (iii) the restriction of the kinetic activity to the chromosomal ends; (iv) the variations in the orientation of bivalents at the division plate, and (v) the possible occurrence of chiasma terminalization. Here we summarize the current knowledge on these topics in the meiosis of Hemiptera (Heteroptera) and present novel results that illustrate some of the special features mentioned above. We also point out the necessity of reviewing the term 'inverted meiosis' and propose some future prospects to study this peculiar meiosis.
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The Meiotic Sequence

Meiosis is one of the most fascinating events in the life of the species with sexual reproduction. This process is characterized by the occurrence of two rounds of chromosome segregation following a single DNA replication S phase, resulting in the production of haploid germ cells. Moreover, due to the existence of recombination events (either as the product of DNA exchange between homologous chromosomes and/or the random segregation of paternal and maternal centromeres during first meiotic division), meiosis promotes the generation of new genetic combinations.

During meiosis, chromosomes perform a series of complex and specific events: homologous chromosomes pair, synapse, recombine and segregate. In yeast, mouse, *Arabidopsis* and grasshoppers both pairing and initiation of synapsis also involve recombination events related to the repair of DNA that takes place in early stages of meiosis. In turn, the correct progression of synapsis, commonly accompanied by a meiosis-specific structure called synaptonemal complex (SC), is necessary for the

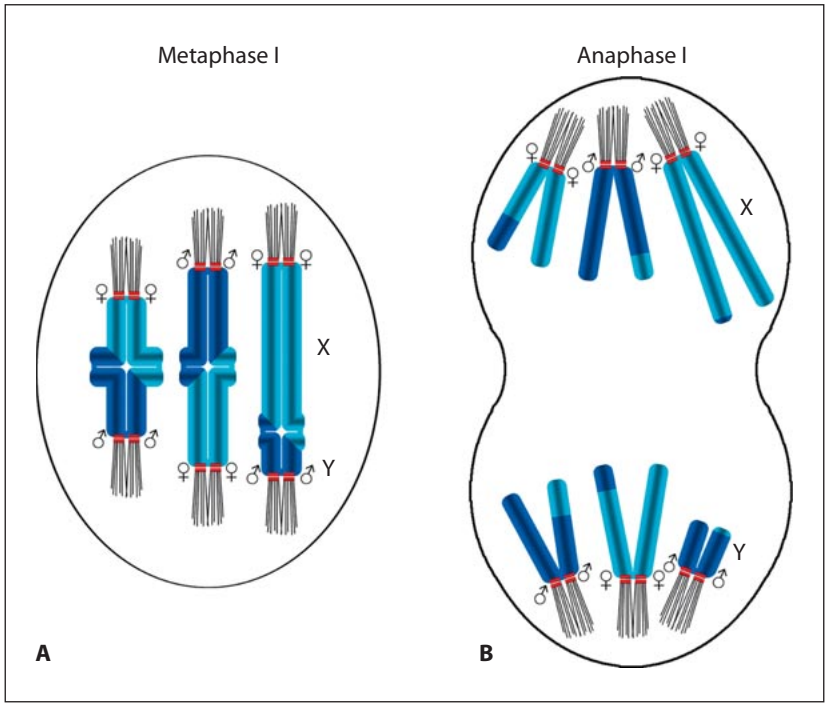

Fig. 1. The canonical meiosis. Drawings of metaphase I and anaphase I cells of a male from a hypothetic species, 2n = 6, XY. Therefore, two autosomal bivalents and a sex bivalent are depicted, all chromosomes present terminal centromere. Maternal and paternal chromosomes are light and dark blue, respectively. Kinetochores and microtubules are represented by two red lines and grey lines, respectively. In each bivalent a single chiasma, subdistal in autosomes and distal in the sex bivalent, is represented. Random orientation (**A**) and segregation (**B**) of paternal and maternal centromeres are also represented. See text for further details.

proper outcome of recombinational events as crossovers, whose cytological manifestations are the chiasmata. Homologous chromosomes appear at the end of first meiotic prophase tightly associated by, at least, one chiasma. However, this association is not provided by the chiasma itself, but by its cooperation, at least, with a family of protein complexes called cohesins that maintain the cohesion between sister chromatids in each chromosome, both at the centromere and along chromosome arms (for review see the Cohesin complexes and sister chromatid cohesion in mammalian meiosis chapter by Suja and Barbero in the present volume). Therefore, sister chromatid cohesion together with reciprocal recombination are responsible for bivalent persistence from late prophase I until homologs segregate at anaphase I.

At metaphase I, bivalents appear stabilized and bioriented at the metaphase plate, that is, each homolog faces an opposite pole. This orientation is ensured by the association of the two sister kinetochores of each homolog, which function co-ordinately to interact with the same pole microtubules (fig. 1A). At the onset of anaphase I, the recombined homologs (in some cases named half-bivalents) separate and move

towards opposite poles, each one with its two chromatids (fig. 1B). The separation occurs after the release of sister chromatid cohesion from chromosome arms at the metaphase I/anaphase I transition. However, cohesion is preserved at centromeres, and hence sister chromatids remain associated until the second meiotic division. In this sense, the kinetochores of maternal and paternal origin segregate reductionally during first meiotic division (fig. 1). At metaphase II, sister kinetochores of each chromosome (half-bivalent) orientate to opposite poles (fig. 2A). During the metaphase II/anaphase II transition, sister chromatid centromere cohesion is lost and then chromatids migrate to opposite poles. Consequently, during this division the sister kinetochores segregate equationally (fig. 2B).

This standard meiotic sequence is characteristic of most sexually reproducing species with monocentric chromosomes, and represents the so called conventional sequence of meiotic segregation or conventional meiosis [1]. However, there are some species in which meiosis is characterized by an inverted sequence because chromosomes segregate equationally during the first meiotic division and reductionally during the second one. This special kind of meiotic division is called post-reductional or inverted meiosis, and is found in some groups of plants and animals [2]. However, the actual occurrence of inverted meiosis is still controversial in some cases [3, 4]. Moreover, in many species autosomes and sex chromosomes can display a different meiotic segregation sequence. This variable behavior has impeded the proper understanding of the causes and consequences of this special kind of meiosis. Notwithstanding, all groups with inverted meiosis share a key feature: they have holocentric chromosomes.

Chromosome Classification as Regards their Kinetic Behavior

Two main categories of chromosomes have been described as regards their interaction with the spindle: monocentric and holocentric. Monocentric chromosomes are those in which spindle microtubules attach to a discrete chromosome region: the primary constriction or centromere. Consequently, the kinetic activity is restricted to that chromosomal region during anaphase segregation. According to this behavior, these chromosomes are also called 'monokinetic'. In contrast, holocentric chromosomes show spindle microtubules attached to the whole or mostly the whole length of the chromatid, thus presenting a non-localized centromere. Therefore, at mitotic anaphase, sister chromatids form two parallel lines during their migration to opposite cell poles and exhibit 'holokinetic' movement (for review, see [2]). In both cases, spindle microtubules interact with kinetochores, but in monocentric chromosomes the kinetochore is solely organized at the centromeric region, whereas in holocentric chromosomes the kinetochore extends along most of the length of sister chromatids.

Holocentric chromosomes are known from plants, animals and protoctists [5]. In plants, holocentric chromosomes are characteristic of Juncaceae and Cyperaceae, and

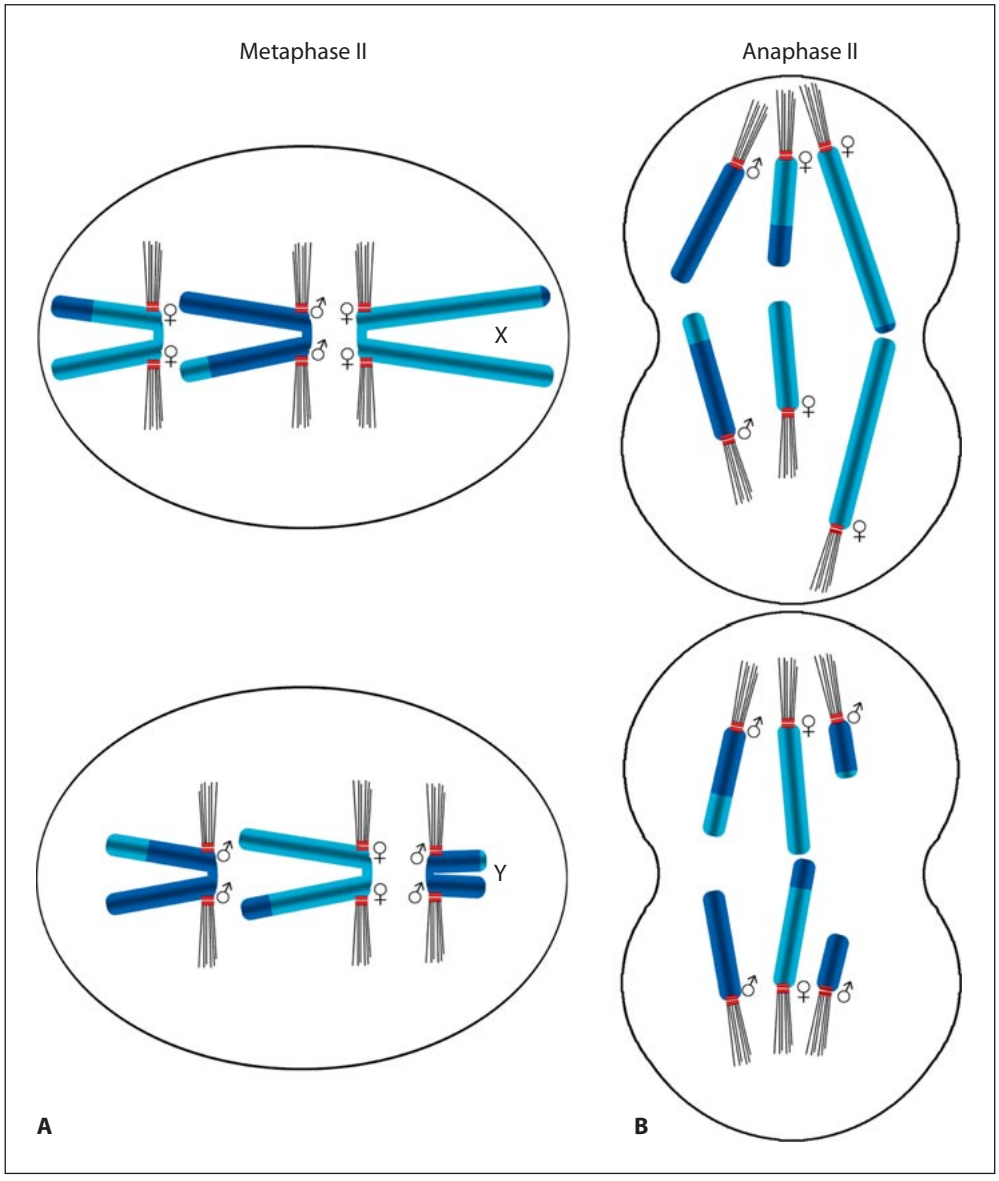

Fig. 2. The canonical meiosis. Second meiotic division in the same hypothetic species represented in figure 1 following the proposed segregation at anaphase I. **A** The two metaphase II cells arising from the first meiotic division are represented. Metaphase II half-bivalents present their sister kinetochores facing opposite poles. **B** Chromatid segregation in anaphase II. See text for further details.

also have been proposed to represent the ancestral chromosome organization in Gramineae [6]. In animals, holocentric chromosomes are present in Nematoda, Arachnida and several insects like Odonata (dragonflies) and Hemiptera (bugs).

The meiotic behavior of holocentric chromosomes has been particularly well documented in heteropteran (true bugs) species [7]. This group is also characterized by

presenting a great variety of sex chromosomal systems: XX/XO; XX/XY and multiple sex chromosomes [7]. In the following sections we mainly focus on the analysis of the chromosomal features of *Graphosoma italicum* (Hemiptera, Heteroptera) (fig. 3A), a species frequently found in Mediterranean forests and that illustrates the characteristic meiotic behavior of heteropterans [8].

Meiotic Chromosome Behavior

The chromosome number of *G. italicum* is 14 (12 autosomes plus XY♂/XX♀) (fig. 3B). The identification of particular chromosomes is extremely difficult owing to the absence of either primary or secondary constrictions. Furthermore, chromosomes appear highly condensed and fused into a single mass of chromatin at mitotic metaphase (fig. 3C). This peculiar chromatin organization is commonly found in species with holocentric chromosomes [9].

In Heteroptera, conventional staining is not useful for determining precisely the sequence of stages throughout the first meiotic prophase. Fortunately, immunolabeling provides a reliable method for the comparison between the classical images and the immunolabeled spermatocytes [10, 11]. The analysis of the cohesin axis throughout the meiotic prophase I has allowed us to correlate the meiotic stage as regards the synapsis progression and chromatin organization [10]. Therefore in conventionally stained spermatocytes, a proper interpretation of the morphological changes that take place in the nucleus during the different meiotic stages could be fulfilled (fig. 3). Using this comparative procedure, some interesting features of chromosome structure and behavior arise: leptotene cells are characterized by a uniform appearance of the chromatin in the nucleus and the absence of any heteropycnotic mass which may represent sex chromosomes (fig. 3D). Consequently, the claimed positive heteropycnosis of the sex chromosomes occurring from the onset of meiosis should be revised. From zygotene onwards, the chromatin of sex chromosomes is differentiated from that of autosomes, due to its positive heteropycnosis (fig. 3E–J). Diffuse stage follows pachytene, although conventional staining techniques based exclusively on chromatin morphology do not discriminate this phase. In the diffuse stage chromatin is less compacted and the nuclei are larger than those of previous stages (fig. 3G). The recondensation of autosome chromatin marks the end of the diffuse stage (fig. 3H) and the beginning of diplotene (fig. 3I). At late diplotene autosomal rod bivalents, and less frequently ring bivalents, are observed (fig. 3J). These two configurations correspond to the presence of one or two chiasmata located at the ends of the bivalents, respectively. At diakinesis the chromatin condensation increases. Thus, the number and the relative size of the autosomal bivalents are determined properly (fig. 3K). Occasionally, bivalents with an interstitial chiasma are observed (asterisk in fig. 3K; see below for further details).

Sex chromosomes present a different and particular behavior of chromatin condensation throughout meiotic prophase stages. As mentioned above, sex chromosomes

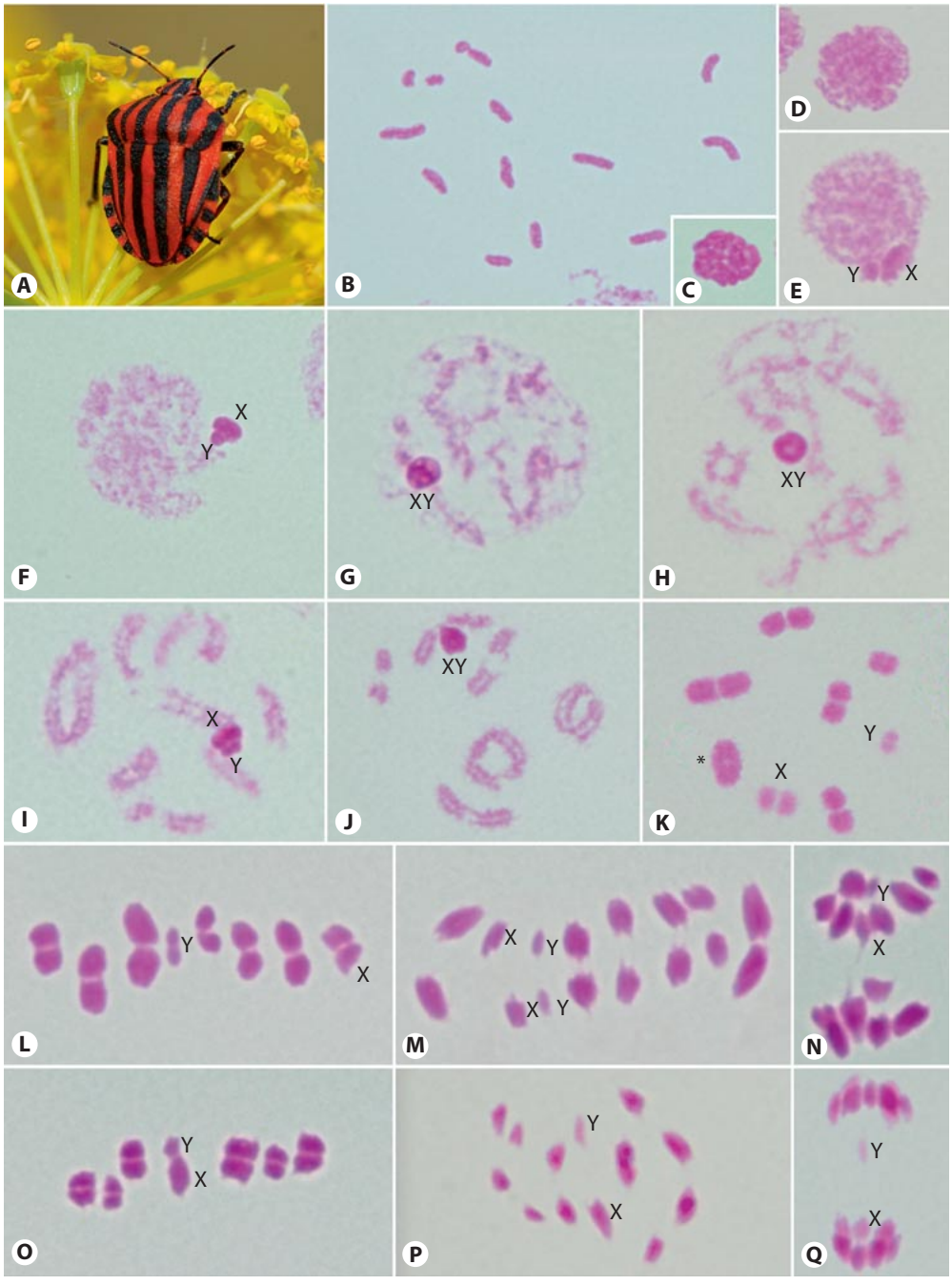

Fig. 3. Germ line cells of *Graphosoma italicum* after Feulgen's staining. **A** *G. italicum* male specimen. **B** Spermatogonial prometaphase with 2n = 14. Notice that neither primary nor secondary constrictions are observable. **C** The aggregation of all chromosomes into a single mass in spermatogonial metaphases impedes their precise identification. **D**–**Q** Spermatocytes. **D** Leptotene/zygotene: chromatin appears uniformly condensed. **E** Early pachytene: sex chromosomes (X and Y) are clearly individualized. **F** Mid-pachytene: sex chromosomes are coupled into a single heteropycnotic mass. Notice that this association is maintained until diplotene (**I** and **J**). **G** Diffuse stage: the nucleus is

become visible in the nucleus at the end of zygotene. At early pachytene X and Y chromosomes are still individualized in the nucleus (fig. 3E). Subsequently, sex chromosomes get closer in mid/late-pachytene (fig. 3F), albeit their cohesin axes do not contact [10]. From this stage until diakinesis, sex chromosomes are associated into a single heteropycnotic mass (fig. 3G–J). In diakinesis two main changes are observed in the sex chromosomes: i) they separate and appear as univalents with their sister chromatids associated; and ii) their heteropycnotic pattern changes from positive to negative (fig. 3K).

At metaphase I autosomal bivalents are aligned at the equatorial plate with their longitudinal axes parallel to the polar axis, whereas the X is orientated with its longitudinal axis perpendicular to the polar axis (figs. 3L and 4A). Interestingly, at prophase I/early metaphase I some bichiasmate bivalents are present, but at late metaphase I only autosomal monochiasmate bivalents with the chromosomal ends without chiasmata facing opposite poles are observed (figs. 3L and 4A). This reduction in visible chiasmata between early and late metaphase I has been classically interpreted as chiasma terminalization [12].

Chromosomes initiate the migration to the poles at the onset of anaphase I. At this stage the kinetic activity is not distributed along the chromosomes, but is restricted to the chromosomal ends facing the poles in both the autosomal half-bivalents and the sex chromatids. Autosomal half-bivalents segregate to opposite poles with the sister chromatids joined. However, sex chromosomes separate their chromatids, so that each pole will contain one X and one Y chromatid (fig. 3M). Therefore, sex chromosomes behave equationally during the first meiotic division. At anaphase I/telophase I (fig. 3N) the X and Y single chromatids associate showing the denominated 'touch and go pairing' [13]. The second meiotic division occurs without interkinesis (data not shown), and at the second metaphase, autosomal half-bivalents with tightly associated chromatids orientate with their longitudinal axis perpendicular to the polar axis. This situation is more evident in the larger autosomes of the chromosome set (fig. 3O). On the other hand, X and Y chromatids are associated and with their long axes parallel to the polar axis, forming a particular heteromorphic structure called pseudobivalent. While associated

bigger and shows a different chromatin condensation if compared to earlier stages. **H** Early diplotene: chromatin recondensation begins. **I** and **J** Diplotene: the nucleus shows 6 individualized autosomal bivalents and associated positively heteropycnotic sex chromosomes. **K** Diakinesis: sex chromosomes become individualized and show negative heteropycnosis. Chromatin condensation increases notably. Asterisk marks an autosomal bivalent with an interstitial chiasma. **L** Metaphase I: the six autosomal rod-shaped bivalents and the X and Y univalents are located at the division plate. **M** Anaphase I: the autosomal half-bivalents, and the sister chromatids of sex chromosomes segregate with kinetic activity restricted to one of the chromosomal ends. **N** Telophase I: the half-bivalents and sex chromatids reach the cell poles. **O** Metaphase II: autosomal half-bivalents and the X and Y chromatids, showing a pseudopair structure, orientate at the division plate. Notice the perpendicular orientation of all the autosomes as regards the polar axis. **P** Anaphase II: segregating chromatids show the kinetic activity restricted to one of their chromosomal ends. **Q** Telophase II: the reductional segregation of sex chromatids is clearly observed. The position of individual sex chromosomes (X and Y in **E, F, K**–**Q**), and the sex chromatin (XY in **G**–**J**) are indicated.

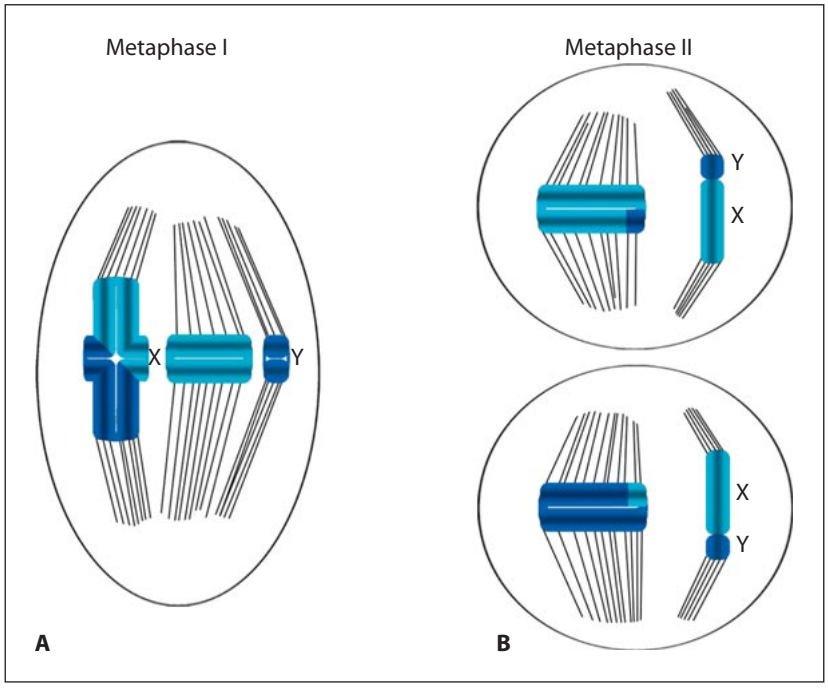

Fig. 4. The inverted meiosis. Drawings of metaphase I and metaphase II cells of a male from a hypothetic species, 2n = 4, XY. Therefore, a single autosomal bivalent and the sex univalents are represented. Maternal and paternal chromosomes are light and dark blue, respectively. Microtubules are depicted as grey lines. A single subdistal chiasma is represented in the autosomal bivalent. **A** At metaphase I the bivalent orientates with the longitudinal axis parallel to the polar axis, while the X univalent is oriented with its longitudinal axis perpendicular to the polar axis. **B** At metaphase II the autosomal half-bivalent is with its longitudinal axis perpendicular to the polar axis. Sex chromatids (X and Y) arranged into the pseudopair structure have their long axes parallel to the polar axis. See text for further details.

chromatids of the autosomes lay in parallel to the metaphase II plate, during anaphase II the kinetic activity is again restricted to the chromosomal ends (fig. 3P–Q). At this stage, X and Y chromatids segregate to opposite poles. Therefore, the second meiotic division is equational for autosomes and reductional for sex chromosomes.

Whence an Inverted Meiosis?

Considering the distinctive features of chromosome segregation during meiosis in *G. italicum* and other heteropteran species, it becomes evident that autosomes seem to follow a conventional sequence of segregation (pre-reductional) while sex chromosomes present inverted or post-reductional meiosis. This pattern differs from that shown by the vast majority of organisms in which both autosomes and sex chromosomes segregate reductionally during the first meiotic division (compare fig. 1 and 4).

But, what characterizes the inverted segregation sequence? Three main features may be determinant: (i) sex chromosomes appear as univalents at metaphase I; (ii) sex chromosomes and all the other chromosomes in the complement, lack localized centromeres, and (iii) all the chromosomes lack an organized kinetochore structure during meiosis. Thus, sex univalents will show amphitelic orientation at metaphase I, and their sister chromatids will migrate to opposite cell poles, as they do in mitosis. This behavior contrasts with that of natural univalents present in species with monocentric chromosomes (e.g.: the single X chromosome of males in Orthoptera). In these cases, the X usually segregates reductionally due to the coordinate association of sister kinetochores to achieve a syntelic orientation. On the contrary, the absence of a differentiated kinetochore in holocentric chromosomes might complicate the coordinated orientation of sister chromatids to the same cell pole. Furthermore, localized centromeres of monocentric chromosomes promote the association of a set of proteins that prevent cohesion degradation by the separase at anaphase I, thus allowing sister chromatids to remain associated until metaphase II [14, 15]. In contrast, the absence of a localized centromere in holocentric chromosomes could allow separase to degrade cohesins all along the chromosome promoting their complete separation at anaphase I.

On these grounds, one would expect that all univalents of holocentric chromosomes show inverted meiotic segregation. Studies in Hemiptera, both heteropterans and homopterans, have revealed that indeed this is the case [16–18]. However, it has been suggested that the segregation of univalents and the single X chromosome, during male meiosis of *Caenorhabditis elegans*, is reductional in the first meiotic division [19, 20]. Albeit direct proof for this suggestion is lacking, this could indicate that the regulation of univalent segregation may differ between different organisms. In this sense, the association of some kinetochore-related proteins on the meiotic chromosomes in *C. elegans*, has been demonstrated, and consequently a kinetochore-like structure is possibly assembled during meiosis in this species [21].

While the inverted meiotic sequence is out of doubt for univalent chromosomes, it is more controversial whether bivalents follow that behavior. Several authors have claimed, based on the interpretation of the orientation of bivalents at metaphase I, that post-reductional segregation occurs in some species of homopterans, dragonflies and plants [2, 3, 17]. In this sense, two main orientations of bivalents have been considered. The first one refers to the morphology of chromosomes shown in fig. 3L (see also fig. 5A, A'), in which there is a distal chiasma holding homologs, and this rod-shaped bivalent orientates with its long axis perpendicular to the cell equator. This orientation, also called co-orientation [2], presumably should lead to a pre-reductional or conventional segregation. The second orientation also refers to rod-shaped bivalents, but that are orientated with their long axis parallel to the cell equator (fig. 5B, B'). This mode of orientation is called auto-orientation and presumably should lead to a post-reductional segregation [2, 3, 17]. However, we have previously demonstrated that this supposition is completely erroneous [22]. For this purpose, we

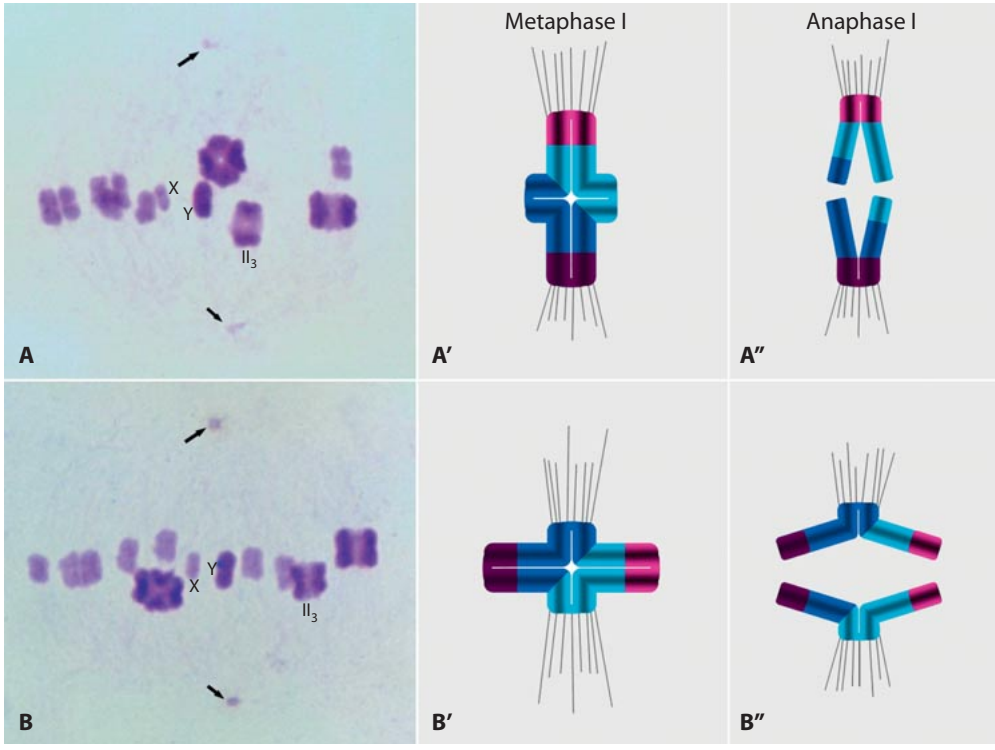

Fig. 5. The autosome segregation in *Triatoma infestans*. **A** and **B** Metaphase I after C-banding. Heterochromatic bands are located at the ends of distinct bivalents. Homologous chromosomes are either light or dark blue. The heterochromatic bands are purple and the microtubules are grey lines. **A'**, **A"**, **B'** and **B"** The two orientation and segregation alternatives for the bivalent 3 (II_3) are represented. In **A**–**A"**, the orientation and segregation correspond to the heterochromatic regions because these are the chromosomal ends with kinetic activity, whereas in **B**–**B"** those roles are exerted by the euchromatic chromosomal ends. The position of individual sex chromosomes (X and Y in **A** and **B**) is indicated. See text for further details.

analyzed the meiotic segregation in the heteropteran *Triatoma infestans* which possesses a chromosomal marker useful for discerning the segregation behavior of chromosomes (fig. 5). In this species, autosomes can present a chiasma close to a chromosome end and thus show a rod-like configuration. Interestingly, these bivalents may orientate either perpendicular (fig. 5A) or parallel (fig. 5B) to the cell equator, depending on which chromosomal end presents kinetic activity. Nevertheless, in any of these two cases, there is a part of the half bivalents, that, extending from the chiasma position up to the kinetic end, segregates reductionally, while the segment comprised between the chiasma region and the non-kinetic end segregates equationally (fig. 5A" and B"). This situation is analogous to the segregation of monocentric chromosomes, in which the centromeres always segregate reductionally, while the segments distal to the chiasma do it equationally (fig. 1). The main difference is that

in *T. infestans* a given chromosome segment will segregate equationally or reductionally depending on which of the two chromosome ends directs the segregation of the half-bivalents at anaphase I.

We can conclude that since there is always a chromosome segment that reduces during the first meiotic division (the kinetic chromosomal end), it makes no sense to consider that chiasmate chromosomes follow the inverted meiosis pattern. In our opinion, the claim that this concept can be maintained from a cytological point of view [17] is untenable. In consequence, the inverted status of the meiotic process in species with holocentric chromosomes must be revised [4, 22, 23] and, in any case, to be necessarily restricted to achiasmate chromosomes.

Kinetic Restriction at the Chromosomal Ends

One fascinating peculiarity shown by heteropteran chromosomes is the differential kinetochore assembly during mitosis and meiosis. Although mitotic chromosomes display kinetochores on most of the length of their chromatids and segregate in a holokinetic manner at anaphase, during meiosis these chromosomes present a restriction of the kinetic activity at the chromosomal ends, thus behaving as 'telokinetic'. This kinetic restriction was elegantly shown in large autosomal bivalents of sectioned material stained with Heidenhain's haematoxylin in the pentatomid *Rhytidolomia senilis* [24]. This finding was later supported ultrastructurally by the absence of detectable kinetochore structures in meiosis [8, 25–27]. The absence of kinetochore and the restriction of kinetic activity have remarkable consequences for the behavior of holocentric chromosomes during meiosis. One of the most extraordinary features is that both ends of the chromosomes can acquire kinetic activity [19, 25, 27]. As mentioned above, this opens the possibility that a given bivalent could achieve two alternative orientations at the metaphase I plate, but also has other consequences as regards the chromosome behavior that we will discuss separately.

Kinetic Restriction at the Chromosomal Ends: Sex Chromosomes

Chromosome analyses in Heteroptera have revealed that although there are different sex chromosomal determination systems, sex chromosomes segregate equationally during the first meiotic division and reductionally during the second one [7], with a few exceptions [28]. In *Graphosoma italicum* the chromatids of the X chromosome are associated all along their length until the first meiotic anaphase, and orientate at the metaphase I plate with their long axes perpendicular to the polar axis (fig. 6). This peculiar orientation is evident by both the position of the chromosome at the metaphase plate (fig. 6A and C), and the interaction with the spindle microtubules (fig. 6B and C'). Microtubule bundles interact with the entire length of both sister chromatids which face different cell poles (fig. 6). Thus, holokinetic interaction

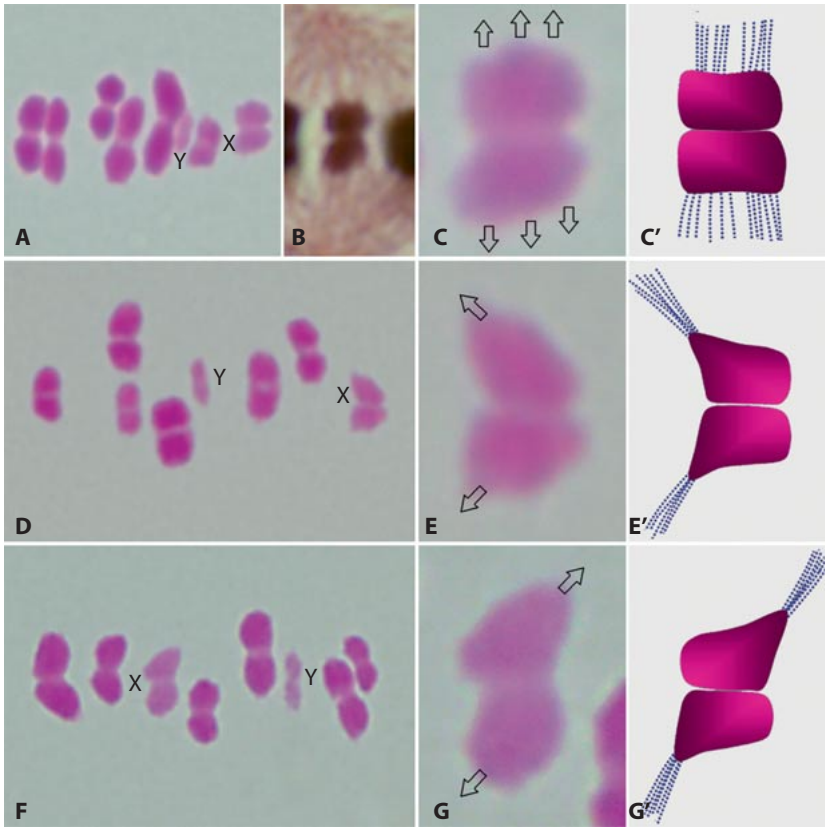

Fig. 6. The X chromosome in *Graphosoma italicum*. **A** Early metaphase I: the X chromosome orientates with its long axis perpendicular to the polar axis. **B** Microtubule bundles show interactions with the entire length of the chromatids giving rise to the holokinetic orientation (**C**). **C′** Schematic representation of this type of orientation at early metaphase I. **D**–**G′** Late metaphase I: the kinetic activity is restricted to either the same (**E**) or opposite (**G**) chromosomal ends of the sister chromatids. Thus, the interacting microtubules can attach to either the same (**E′**) or different (**G′**) chromosomal ends. The positions of individual sex chromosomes (X and Y in **A**, **D** and **F**) are indicated.

between the spindle and the X chromatids is the most plausible mechanism for the stabilization of the X chromosome at the metaphase I plate. However, at late metaphase I the kinetic activity becomes restricted to the chromatid ends. Our previous reports showed that the NOR is located at one of the chromosomal ends of the X. Thus, we could demonstrate that both chromatid ends are able to develop kinetic activity. Moreover, the restricted kinetic activity is a random process [9, 29], and the election of the kinetic end is independent between sister chromatids. Thus, the X chromosome can adopt two distinct morphologies: (i) the same chromatid end in both sister chromatids acquires kinetic activity (fig. 6D, E'), and (ii) the kinetic activity is located at one end in one chromatid and at the opposite end in the sister chromatid (fig. 6F, G').

Therefore, the initial holocentric interaction of the X chromosome with the spindle becomes a monokinetic interaction at late metaphase. This is randomly elected between the chromosomal end and is achieved independently in each sister chromatid. It remains to be answered how the spindle interaction changes and how and why the kinetic ends are selected. Since this selection is a random process, the restriction of kinetic activity to a specific chromatid end is not determined on the X chromosome prior to metaphase I.

Kinetic Restriction at the Chromosomal Ends: Autosomes

The interaction of autosomes with the meiotic spindle mostly depends on the morphology (either rod or ring) of each bivalent. In heteropterans most autosomal bivalents present a rod-like configuration that has been correlated with a single distal chiasma. In this case, bivalents usually orientate at metaphase I with their long axes parallel to the polar axis (fig. 5A') (previously mentioned as co-orientation), but it is also possible that the bivalent has its long axis parallel to the cell equator (fig. 5B'). This refutes the proposal that the end showing kinetic activity during the first meiotic division is neither involved in nor close to the chiasma [30]. Nevertheless, in both kinds of orientations the kinetic activity is restricted to the chromatid end that faces the poles, and relevantly, in both homologs to the same chromosome end. As occurs with the sex chromosomes, both chromosome ends are able to show kinetic activity (fig. 5). However, a single end never shows kinetic activity during both divisions. On the contrary, those ends presenting kinetic activity at anaphase I are inactive at metaphase II and vice versa [22, 31].

In many heteropteran species bichiasmate bivalents are present. In this case, it is observed that homologous chromosomes are associated at both ends and present a ring configuration during diplotene and diakinesis. In some species, as in the heteropteran *Largus rufipennis*, bivalents with two apparent chiasmata congress to the metaphase I plate showing holokinetic behavior, but then one chiasma seems to resolve before anaphase [16]. Likewise, in bichiasmate bivalents of *G. italicum* the longitudinal chromatid axis is perpendicular to the polar axis from prometaphase I up to early metaphase I (fig. 7A–B'). However, bichiasmate bivalents show a well defined sequence of morphological changes during metaphase I. From early (fig. 7A–B') up to mid metaphase I (fig. 7C and C') the homologous chromosomes separate at one chromosomal end and only remain associated in the region occupied by the second chiasma (fig. 7C and C'). At late metaphase I, all bivalents show a single chiasma and are aligned with their long axis perpendicular to the cell equator (fig. 7D and D'). Old observations in the pentatomid *Rhytidolomia senilis* (see figures in [24]) may correspond to the early resolution of one of the chiasmata of a bichiasmate bivalent (see the next section for further information). It remains to be answered whether in bichiasmate bivalents the election of the kinetic end is at random, as occurs in the X chromosome. Nevertheless, the kinetic activity is undoubtedly restricted to the same end in both homologous chromosomes.

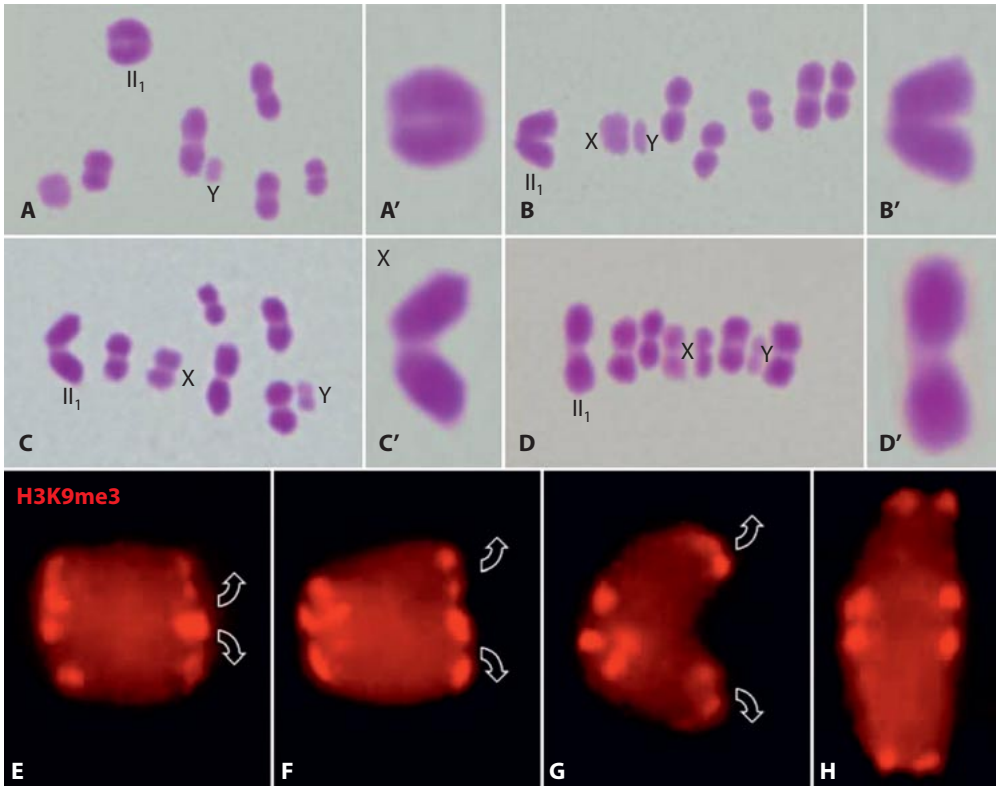

Fig. 7. Bichiasmate bivalent segregation. First meiotic metaphases of *G. italicum* after Feulgen staining (**A–D′**) and H3K9me3 immunolabeling (**E–H**). **A** Early metaphase I: a single bivalent presenting two chiasmata (II1) is observed. Notice that the main axis of this bivalent is perpendicular to the polar axis (**A′**). **B** and **C** Mid metaphase I: open bivalents (II1) are observed. **B′** and **C′** In these cases, the kinetic activity is restricted to the same end of each chromosome. **D** Late metaphase I: all bivalents show distal associations. **D′** Enlargement of the longest bivalent. **E**–**H** Sequence of the resolution of a bichiasmate bivalent throughout metaphase I as seen with H3K9me3 immunolabeling. Selected bivalents from stages that correspond to those of figures **A**–**D** are shown. That is: early (**E**), mid (**F** and **G**) and late (**H**) metaphase I. In all these bivalents eight H3K9me3 signals, one in each chromatid end, are observed. The position of individual sex chromosomes (X and Y in **A**, **B**, **C** and **D**) is indicated. See text for further details.

New Markers to Solve Old Problems: Chiasma Terminalization

Although several studies have carefully analyzed meiotic divisions in a variety of species presenting inverted meiosis, the hypercondensation of chromosomes has impeded an accurate analysis on the number and position of chiasmata in metaphase I bivalents. Interstitial chiasmata are observed in diplotene and diakinesis, but when chromosomes condense at first meiotic division they usually acquire a rod-like morphology in which homologous chromosomes seem to maintain an end-to-end association. This observation has been interpreted as chiasma terminalization events. This hypothesis implies that a chiasma moves during late prophase I from the site of its generation up to the

bivalent end by metaphase I [32]. Terminalization has clearly been refuted in species with monocentric chromosomes [33] and also in hemipterans with large chromosomes, like *T. infestans* [22] and *Myrmus miriformis* [23]. However, this is not so obvious in hemipteran species with hypercondensed small chromosomes and rod-shaped bivalents at metaphase I because of the lack of reliable cytological markers. Fortunately, immunolabeling techniques allow to partially solve this question [10, 34]. We have performed a survey for new chromosomal markers that could shed light on the nature of chromatin organization in hemipterans, and found that some histone variants, mainly histone H3 trimethylated at lysine 9 (H3K9me3), are specifically accumulated at the chromosomal ends in *G. italicum*. H3K9me3 is an epigenetic indicator which is recognized by the heterochromatin binding protein 1 (HP1), and seems to be essential for a proper heterochromatin arrangement [35, 36]. Moreover, during male mouse meiosis the methylation of lysine 9 in H3 is involved in the process that leads to the accurate centromere clustering at the onset of meiosis. At early meiotic stages methylated H3K9 distributes over the chromatin, and recruits at both the chromocentres, which represent clustered autosomal centromeric regions, and the XY body during pachytene and diplotene. At diakinesis, the modified H3 persists specifically located in the pericentric heterochromatin of both autosomes and the sex chromosomes [37].

In squashed pachytene spermatocytes of *G. italicum*, H3K9me3 is located in a particular nuclear region close to the nuclear envelope at the bouquet basis (fig. 8A–C). Although both sex chromosomes are profusely stained, H3K9me3 labeling seems to extend to certain autosomal regions (fig. 8A–C). In order to locate precisely these regions we analyzed spread pachytene spermatocytes (fig. 8D and E) in which histone H3K9me3 is located at both ends of the autosomal cohesin axes, as revealed by the location of the SMC1α protein (fig. 8D and E). At metaphase I, H3K9me3 labels the entire length of both the X and the Y chromosomes as well as discrete domains of autosomal bivalents (fig. 8F). Interestingly, in each metaphase I autosomal bivalent the signals appear as individualized spheres at the chromosomal ends (fig. 8F–H). As a rule, eight H3K9me3 signals are clearly discerned. These appear in couples as a consequence of the association of sister chromatids (fig. 8F–I). This labeling pattern allows to discern different situations in the hypercondensed metaphase I bivalents of this species. Thus, bivalents with a single chiasma are rod-shaped and display their longitudinal chromosome axis parallel to the pole axis at metaphase I, regardless of the position of the chiasma. Moreover, they show a pair of H3K9me3 signals at the ends that face the cell poles, whereas four signals are located close to the region of the chiasma at the division plate. Interestingly, monochiasmate bivalents can be divided into two main categories: (i) bivalents in which signals appear intimately associated and that we interpret as presenting a single distal chiasma (fig. 8F and G), and (ii) bivalents in which the signals appear separated and that we consider to have a sub-distal chiasma (fig. 8F, H and I). The latter, which is due to the existence of a chromosomal region beyond the chiasma position, strongly supports the existence of pseudo-terminalization [12, 38] in these chromosomes [22]. Thus, subdistal chiasmata are reliably

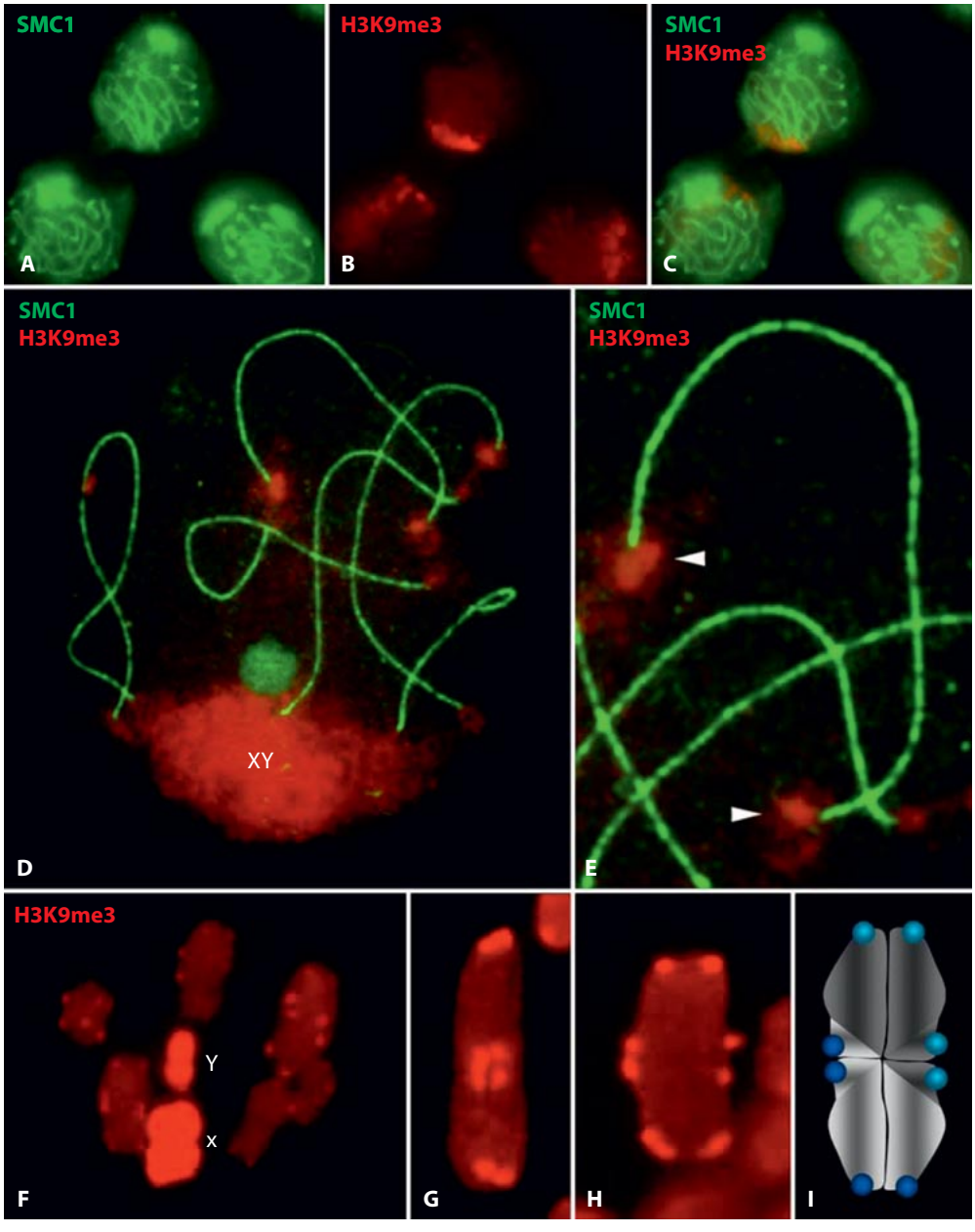

Fig. 8. Protein immunolabeling in *G. italicum*. SMC1α (green) and H3K9me3 (red) location in squashed (**A**–**C** and **F**–**H**) and spread spermatocytes (**D** and **E**). **A**–**C** Pachytene: SMC1α labels both the autosomal and the sex chromosomes' cohesin axes (**A**), whereas H3K9me3 labels a large area (**B** and **C**) close to the SMC1α bouquet basis. **D** Spread pachytene and **E** selected autosomal pachytene bivalent in which the H3K9me3 labeling is observed in the sex chromosomes (XY) and both ends of every autosomal cohesin axis. **F** Metaphase I: the sex chromosomes (X and Y) are thoroughly stained by the anti-H3K9me3 antibody, and pairs of H3K9me3 signals are distinguishable in the autosomal bivalents' ends. **G** and **H** Two selected metaphase I bivalents showing either a distal (**G**) or a sub-distal (**H**) chiasma. **I** Schematic representation of a metaphase I bivalent with a

detected by both classical methods (asterisk in fig. 3K), and the immunolocalization of the chromosomal tips in hypercondensed metaphase I bivalents (fig. 8F, H and I). These observations do not allow to discern whether a chiasma generated in terminal regions of the chromosomes either does or does not terminalize. However, some bivalents present subdistal chiasmata at metaphase I. Hence, one can confidently conclude that these chiasmata do not terminalize in the classical sense [32].

Ring bivalents were also analyzed with these cytogenetic tools. In this case, the first question that arises is to determine the chiasmatic nature of the association at the two chromosomal ends. It is not possible to discard that these associations are non-chiasmatic. However, indirect evidences allow us to consider them as chiasmatic: (i) heterochromatin is present in both ends of each chromosome (data not shown). Consequently, achiasmate heterochromatic association should be equally possible. But, in fact, this is not the case; (ii) ring bivalents only occur in the longest bivalents. They have never been observed in the short bivalents, (iii) ring bivalents are present in diplotene/diakinesis and persist in the hypercondensed bivalents of prometaphase/metaphase I, (iv) ring bivalents are stabilized at the metaphase I plate with their longitudinal axes perpendicular to the polar axis and maintain both distal associations (chiasmata). In contrast to what is observed in rod bivalents, ring bivalents show their eight H3K9me3 signals at the metaphase I plate. This is due to both the presence of two unsolved chiasmata, one in each distal end, and the disposition of these bivalents in the metaphase I plate (fig. 7G). However, as mentioned above, bivalents with two chiasmata undergo a process of reorganization at metaphase I [16, 24] (fig. 7A–D'). Thus, as long as two chiasmata are present, sister H3K9me3 signals are associated to the chiasma regions (fig. 7E). Subsequently, throughout metaphase I one of the chiasmata resolves when one of the chromosomal ends acquires kinetic activity (fig. 7F). It must be stressed that this chiasma seems to be resolved without losing sister chromatid cohesion along the entire length of their chromatids, because H3K9me3 signals remain associated at the kinetic tips (fig. 7F–H). Then, the bivalent starts to open, probably due to the spindle tension executed by the microtubules at the kinetic ends, whereas the remaining chiasma works as a 'hinge' (fig. 7F–G). Before anaphase I migration two H3K9me3 signals are observed at the distal tip of the kinetic ends facing the cell poles, whereas those signals which are close to the unsolved distal chiasma appear located at the division plate (fig. 7H). Finally, the bivalent adopts a rod-shaped conformation which is similar to that shown by monochiasmate bivalents (compare figs. 8G–H and 7H).

sub-distal chiasma. Sister chromatids in each homologous chromosome are light and dark grey, respectively. Consequently, light or dark blue spheres mark the position of the H3K9me3 signals at different ends of each chromosome. The position of individual sex chromosomes when their identification is possible (X and Y in **F**), and the sex chromatin (XY in **D**) are indicated. See text for further details.

Future Prospects

Many questions remain unsolved as regards the meiotic chromosome behavior in Hemiptera. Two of them are particularly appealing to us: (i) the identification of the factor(s) involved in the restriction of the kinetic activity to solely one chromosome/chromatid end, and (ii) the regulation of sister chromatid cohesion during both meiotic divisions.

As regards the acquisition of kinetic activity, it is evident that each chromosome end may interact with spindle microtubules during the first meiotic division [9, 22, 29]. In some cases, e.g. monochiasmate bivalents of *G. italicum*, this interaction seems to be influenced by the presence of a chiasma in the opposite end of the bivalent [30]. Indeed, this does not rule when two chiasmata are present. Moreover, the two possible orientations in a given bivalent that are unrelated to chiasma position in *T. infestans* [22] indicate that other factors must be responsible for the acquisition of kinetic activity at a given chromosome end. Interestingly, in two hemipteran species, *Euschistus servus* and *E. tristigmus,* the chromosome fragments produced after irradiation invariably show kinetic activity restricted to their new originated ends [24]. Thus, it is still unknown which proteins/factors are required not only to promote a chromosome end to be kinetically active, but also to determine the specific chromosome region that directs the poleward movement in chromosome segregation. Further analyses which are needed to comprehend this phenomenon will, in turn, disclose interesting features as regards the centromere origin, function and evolution in species with holocentric chromosomes. Testing certain proteins involved in the kinetochore/centromere of mitotic holocentric chromosomes of *C. elegans* (reviewed in [29]), hopefully will reveal some clues for the resolution of this question.

At present we know that monocentric chromosomes lose the cohesion between sister chromatids during metaphase I/anaphase I except in the centromeric region. Then, chromatids lose that cohesion in the metaphase II/anaphase II transition. In contrast, metaphase II half-bivalents of hemipteran species exhibit closely associated chromatids all along their lengths (fig. 3O). Therefore, the pattern of meiotic cohesion release in hemipteran species dramatically differs from that of monocentric species due to both the existence of a large diffuse stage in prophase I and the absence of centromeres. Now we have some results in these holocentric chromosomes as regards the localization of different proteins previously involved in cohesion maintenance. For instance, the synaptonemal complex protein SYCP3 in sex chromosomes is not directly responsible for sister chromatid cohesion [11]. Additionally, some subunits of the cohesin complex such as Rec8 [34], SMC3 [10] and SMC1α (some results are here included and the manuscript is in preparation) have been localized in prophase I spermatocytes of different hemipteran species. Nonetheless, their presence and location in further meiotic stages is still obscure. In conclusion, the distribution of cohesin subunits and other proteins involved in cohesion, will shed some light on the dynamics of sister chromatid cohesion and segregation.

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Author Index

Alsheimer, M. 81

Barbero, J.L. 94

Camerini-Otero, R.D. 117

De Muyt, A. 14

Garcia Caldés, M. 128
Garcia-Cruz, R. 128
Grelon, M. 14

Khil, P.P. 117

Martinez-Perez, E. 26
McFarlane, R.J. 1
McKee, B.D. 56
Mercier, R. 14
Mézard, C. 14

Page, J. 137
Pryce, D.W. 1

Roig, I. 128
Rufas, J.S. 137

Suja, J.A. 94

Viera, A. 137

Wang, P.J. 69

Yang, F. 69

Zetka, M. 43

Subject Index

Aneuploidy 128
Arabidopsis thaliana 15
Axial elements (AE) 70, 98

Bouquet formation 81
Bug 137

Caenorhabditis elegans 43
Central element (CE) 69, 73, 98
Centromere association 30
Cereal crops 26
  genome structure 28
Chiasma
  formation 52
  terminalization 150
Chromatin transition 5
Chromosome
  dynamics 81
  pairing 43
  segregation 1, 43, 94
  structure 137
CO pathways 20
Cohesin 94
  axes 101
  complexes 70, 94
  deficient mice 105
  proteins 70
  regulators 94, 108
Cohesion 99, 137
Control of
  gene expression 110
  pairing 64
CRE hotspot 2
Crossover (CO) 14, 49

Distribution of recombination events 16
DNA binding proteins 2

Drosophila 56
  female germ cells 60
  male germ cells 58
  X-Y pairing 63
DSB
  processing 19
  formation 18, 50
  repair 19

Epigenetics 117

Fission yeast 1
Function of SC proteins 75

Genetic control of pairing 64
Graphosoma italicum 141

Heterochromatin 56, 60
Holocentric chromosomes 137, 139
Homolog
  alignment 44
  pairing 26, 43, 56
  recognition region (HRR) 45
  segregation 54
Homologous recombination 69
Hotspot 1, 117
  activation 3
Human 117, 128

Inverted meiosis 137

Kinetic
  activity 52
  behavior 139
  restriction 147

Lamins 81, 88

Lateral element (LE) 69, 98
Linkage
  analysis 119
  disequilibrium 117, 121

M26 hotspot 2
  activation 3
  chromosomal architecture 3
Maize 26, 35
Mammals 69, 94
Maternal age effect 128, 134
mbs1, -2 9
Meiosis 1, 14, 26, 43, 56, 62, 69, 94, 128
Meiotic
  chromosome behavior 141
  sequence 137
Mitotic pairing 58, 61
MLH1 heterogeneity 128
Mouse mutants 72, 105
M-pal 10
Mutants 6

Nondisjunction 128
Nuclear
  envelope (NE) 81
  lamina 81, 87

Oocytes 128
Oogenesis 128

Pairing centre (PC) 45
Pairing sites 56
Ph1 mutants 31
Plant 14
Polymorphism 117, 123
Pre-meiotic
  DNA replication 8
  nondisjunction 129
Protein interaction 74

Rec12 3
  binding 10
Recombination 1, 14, 26, 43, 49, 117, 128
  hotspots 1, 9, 117
  markers 14
  model 14
  mechanisms 18
  variation 117
Reproduction 14
Rice 26, 33
Rye 26, 37

SC-associated proteins 75
Schizosaccharomyces pombe 1
Segregation 56
Sex chromosomes 137
Shugoshins 94, 109
Sperm genotyping 117, 120
Spermatocytes 128
Spo11 18
Strand invasion 19
Subunits of cohesin complexes 95
SUN-domain proteins 81, 86
Synapsis 43, 69, 128
Synaptonemal complex (SC) 48, 69
  identification 69
  organization 48
  proteins 70, 98
    deficient mice 105
  structure 48

Telomere 81
  attachment 81, 83
  attachment complex 81, 87
  bouquet 26, 30, 81
  clustering 81
  movement 84, 88
Transverse filaments (TF) 69, 73, 99
Trisomy 128

Ultrastructure 83
ura4::aim 9

Variation 117

Wheat 26, 30